TOXICOLOGIST'S REVIEW

PLA #: 98-0261
SPONSOR: SERONO LABORATORIES, INC.
PRODUCT: recombinant, human interferon-β 1a (REBIF®, Chinese hamster ovary cell-derived)
FORMULATION: sterile solution containing HSA, mannitol, and WFI, USP
RELATED DOCUMENTS: BB IND XXXXXXXXXX, BB IND XXXXXXXXXX
PROPOSED INDICATION: treatment of relapsing-remitting multiple sclerosis
ABBREVIATIONS: IFN-β, recombinant, human interferon-beta 1a; CHO, Chinese hamster ovary; PBL, peripheral blood leukocytes; 2',5'-OAS, 2',5'-oligoadenylate synthetase; $t_{½elim}$, elimination half-life; AUC, area under the serum concentrations *vs.* time curve; kD, kilodaltons; NOAEL, no observable adverse effect level; MU, million anti-viral units of activity (by cytopathic effect bioassay); MS, multiple sclerosis; MRI, magnetic resonance imaging; HSA, human serum albumin; LDH, lactic dehydrogenase; LD_{50}, dose which causes lethality in 50% of the animals treated; PBL, peripheral blood leukocytes; CPE, cytopathic effect; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ELISA, enzyme-linked, immunosorbent assay; RIA, radioimmunoassay; NOEL, no observable (pharmacologic) effect level; PEL, dose producing a pharmacological effect which is different from control, but without adverse effect; GD, gestational day

received 2/27/98; assigned 3/15/98; completed 2/23/99

TABLE OF CONTENTS

ABSTRACT:

The safety, biochemical, and pharmacologic activity of IFN-β, derived from CHO cells were evaluated in mice, rats, and cynomolgus monkeys *in vivo*, and in peripheral blood leukocytes derived from humans, mice, rats, rabbits, dogs, and cynomolgus monkeys *in vitro*. In *in vitro* pharmacodynamic assays, only PBL from cynomolgus monkeys were found to exhibit increases in 2',5'-OAS enzyme activity similar to that observed with human cells; a dose-related increase in serum neopterin activity was also observed when PBL from cynomolgus monkeys were incubated with IFN-β *in vitro*, or in serum samples from animals treated *in vivo*. Because of the species specificity in the pharmacodynamic response, *in vivo* pharmacologic, pharmacokinetic, and repeat-dose toxicology testing was conducted in rats and in the cynomolgus monkey. Pharmacokinetic studies in these species demonstrated similar absorption and elimination after either s/c or i/m injection, with a "flip-flop" profile observed in both species following injection of 1 MIU/kg IFN-β. Systemic exposure, as calculated from the C_{max} and $AUC_{0-\infty}$ were increased in a dose-related fashion, was approximately linear although greater than predicted, and were similar for both the i/m and s/c routes. Bioavailability by either route was approximately 25 to 40%. IFN-β has pharmacologic and toxicologic profiles similar to other type I interferons; major findings in cynomolgus monkeys after repeated i/m, i/v, or s/c dosing at 0.25, 0.5, 1, 3, or 10 MIU/kg (0.1, 0.19, 0.37, 1.1, 3.7 µg/kg) of IFN-β included slight increases in rectal body temperature, decreased food consumption and weight loss, slight variations in erythrocyte, platelet, and leukocyte counts, and local irritation and inflammation at the site of injection. There was no apparent dose-relationship of any parameter to the dose of IFN-β administered, and the majority of these changes were only evident during the first two to four weeks of treatment. Histologically, increases in lymphoid hyperplasia, chronic inflammation, and hemorrhage were observed at the injection sites after 4 to 26 weeks of treatment, which were also present in animals treated with the vehicle control. The NOAEL for IFN-β in both rats and in cynomolgus monkeys is 1.0 MIU (0.37 µg)/kg/d for 4 or 13 w, by either i/v or i/m injection. Based on the loss of appetite, subsequent weight loss, and hematologic changes in the female monkeys, the NOAEL of IFN-β administered for 6 months by daily s/c injection is 10.5 µg/kg/d (3 MIU/kg/d). The NOAEL in male monkeys is 35 µg/kg/d (10 MIU/kg/d), given by s/c injection for 6 months. These doses correspond to approximately 70 to 245 times the cumulative weekly dose of 66 µg IFN-β used in the pivotal trial (as calculated for a 60-70 kg human), and 35 to 122 times the cumulative weekly dose of 132 µg. When scaled by total body surface area, these doses are approximately 28 to 100-fold greater than the cumulative human weekly dose of XXXXXXXXXX IFN-β proposed for use in relapsing-remitting multiple sclerosis. Pharmacodynamic markers of interferon activity, including 2 to 50-fold increases in serum neopterin and 2',5'-OAS levels were observed in animals after single doses of IFN-β, but declined after treatment for more than 28 days, and levels returned to baseline after 4 to 13 weeks on study. A loss of detectable IFN-β activity in the serum and development of neutralizing antibody activity was noted at the end of treatment period in all studies, beginning by approximately 4 weeks of treatment. IFN-β was tested for reproductive and developmental toxicity in male and female cynomolgus monkeys as part of the 26-week toxicity study, and was

found to have no effect on serum estradiol levels or menstrual cyclicity at doses of up to 10 MIU/kg/d. In male monkeys, there were no significant, treatment related effects of IFN-β treatment on male fertility, as evidenced by serum testosterone levels, sperm counts, motility, and function (hamster oocyte penetration) at doses of up to 10 MIU/kg/d (35 µg/kg/d). Treatment of pregnant, female cynomolgus monkeys from either GD21-89 pr GD90-150 induced significant abortifacient effects at 0.2 MIU (0.74 µg)/kg/d, with a 67% fetal loss rate when animals were treated late in pregnancy. This dose level is approximately 2 to 4-fold greater than the recommended weekly dose of XXXXXXXXXX or 132 µg (36 MIU) in MS patients, when normalized by either body weight or surface area.

INTRODUCTION:

REBIF® [recombinant human interferon-beta 1a (IFN-β)] is a natural protein that is secreted by genetically engineered, mammalian CHO cells, with an amino acid sequence identical to that of naturally occurring human interferon-β. IFN-β is a single chain, glycosylated polypeptide 166 amino acid residues in length, and with an approximate molecular weight of 22.5 kD.

The intended clinical use of IFN-β is in the treatment of MS, a disease that is characterized by progressive loss of motor and sensory neural functions. Multiple sclerosis is believed to result from an abnormal autoimmune response occurring within the central nervous system, which leads to destruction of the myelin sheaths covering nerve fibers, and a resulting deterioration in nerve signal conduction. Interferons are postulated to produce clinical effects in MS by an immunodulation of the aberrant immune response against the myelin sheath, and by antagonizing the immune activating effects of interferon-γ, which has been demonstrated in pilot clinical studies in MS to enhance exacerbation frequencies. *In vitro*, IFN-β decreases mitogen-stimulated T lymphocyte proliferation and interferon-γ synthesis and secretion.

REBIF® is indicated for the treatment of relapsing forms of MS. In patients with MS, IFN-β has been demonstrated to decrease the progression of physical disability, decrease the frequency of clinical exacerbations, and XXXXXXXXXX. The dose and schedule of REBIF® intended for administration chronically to MS patients is XXXXXXXXXX, which is equivalent to XXXXXXXXXX of antiviral biological activity, given t.i.w. by s/c injection. REBIF® is formulated as a sterile, solution containing human serum albumin (HSA), USP, mannitol, USP and water for injection, USP. For clinical use, the product is provided in pre-filled syringes, containing either 22 or 44 µg IFN-β per 0.5 ml. The IFN-β used for all preclinical pharmacology, pharmacokinetics, and toxicology studies was produced at commercial scale, was greater than 99% pure, was formulated according to clinical procedures and was either of clinical grade, or representative of that used in the clinic.

PRECLINICAL PHARMACOLOGY AND PHARMACOKINETICS:

Pharmacology Study Summary:

1. Interferon beta modulation of cytokines in the central nervous system of SJL/J mice with EAE. Study #XXXXXXXXXX. Female SJL/J mice, 10-13 wks old, 0, 75, 150, 300 IU/g purified murine IFN-b, q.o.d. x 21 d (XXXXXXXXXX, lot number not specified); non-GLP; XXXXXXXXXX.

2. Search for pharmacodynamic markers in animal models for toxicological studies. Study #XXXXXXXXXX. Peripheral blood mononuclear cells from mouse, rat, rabbit, dog, marmoset, cynomolgus monkey, and human cells, exposed *in vitro* to IFN-β, lot #XXXXXXXXXX; cynomolgus monkeys, 0, 3.5, 10.5, and 35 mg/kg/d IFN-b, lot #XXXXXXXXXX (0, 1, 3, 10 MIU/kg/d, from toxicology study #XXXXXXXXXX, below); GLP; 5/31 – 7/8/95 (*in vitro* studies), 10/24/95 – 1/4/96 and 9/30/96 – 1/14/97 (*in vivo* studies); XXXXXXXXXX.

Pharmacology Study Review:

Study #XXXXXXXXXX. *Interferon beta modulation of cytokines in the central nervous system of SJL/J mice with EAE.*

Data were presented as a manuscript, submitted for publication in the open literature[1], from a study conducted in an independent, academic laboratory. This experiment used homologous, purified murine IFN-β to evaluate the activity of the biologic in an experimental animal model of multiple sclerosis. A brief synopsis of the data is presented, below.

Experimental, autoimmune encephalitis (EAE) was induced in female SJL/J mice by s/c injection of an emulsion of XXXXXXXXXX myelin basic protein from bovine brain, heat-killed XXXXXXXXXX, and XXXXXXXXXX incomplete adjuvant in XXXXXXXXXX, followed by i/v injection with XXXXXXXXXX toxin on days 0 and 2 post-immunization. Interferon treatment was begun either immediately after immunization with the bovine protein, or after the onset of EAE. Three mice per group were sacrificed at various time points after induction of EAE, and samples of brain tissue were analyzed for expression of various cytokine mRNAs by RT-PCR and PCR assays, and histologically for evidence of inflammatory response.

At doses of 150 IU/g IFN-β and above, onset of EAE clinical signs was significantly delayed from mice treated with either control or 75 IU/g IFN-β. Histologically, however, there was no difference observed in either the incidence or severity of the inflammatory infiltrates present in brain sections from control or IFN-β-treated mice at any dose or time point sampled. Analysis of cytokine mRNA levels by semi-quantitative RT-PCR

[1] Puerta, C., I. Martinez, A. Moreno, M.L. Vaello, and A. Garcia-Merino. 1998. Interferon-beta modulation of cytokines in the central nervous system of SJL/J mice with EAE. *submitted for publication.*

revealed the IFN-β treatment was associated with a down-regulation of expression of the pro-inflammatory cytokines tumor necrosis factor-α, lymphotoxin, interferon-γ, and transforming growth factor-β as compared to control EAE mice, while expression of interleukin-2 and interleukin-10 was unchanged between the groups. These data suggest that one possible mechanism for the activity of IFN-β in MS may be down-regulation of pro-inflammatory mediators, including these as well as other Th_1 lymphocyte-derived cytokines.

Study #XXXXXXXXXX. ***Search for pharmacodynamic markers in animal models for toxicological studies.***

The effects of IFN-β on the pharmacodynamic markers 2',5'-OAS, β2-microglobulin, neopterin, and pro-inflammatory cytokine synthesis were evaluated using PBL from several different animal species, to determine the species demonstrating suitable response for use in further pharmacologic and toxicologic testing. Peripheral blood leukocytes were isolated by XXXXXXXXXX centrifugation of 5 ml samples of whole blood obtained from mice, rats, rabbits, dogs, marmosets, cynomolgus monkeys, and humans. dogs, and Rhesus and cynomolgus monkeys. Isolated PBL were cultured for XXXXXXXXXX in the presence of 0, 50, or 500 U/ml rhIFN-β 1a at a cell density of XXXXXXXXXX, washed, and samples of supernatant fluid assayed for neopterin and β2-microglobulin by commercially available radioimmunoassays. 2', 5'-OAS enzyme activity was determined following the synthesis of 2', 5'-oligoadenyl-5'-triphosphate in treated cell lysates, and was detected by commercial radioimmunoassay. Cytokine synthesis and secretion was determined from samples of culture supernatant fluids taken after XXXXXXXXXX of exposure of PBL from the various species to rhIFN-β or vehicle control, and were measured using commercially available ELISA test kits, specific for either the murine or the human homologues of the protein.

A dose-related increase in the amount of 2',5'-OAS activity after IFN-β1a stimulation was noted in only in cells obtained from human donors. Although an apparent increase in 2', 5'-OAS activity was noted in PBL obtained from beagle dogs, the high background levels of enzyme activity in the control cells suggest that this effect is artifactual (please see Comment, below). No significant stimulation of 2', 5'-OAS activity by rhIFN-β was seen in the rabbit, rat and mouse. Of interest, no stimulation of enzyme activity was noted in PBL obtained from the two non-human primate species, marmosets and cynomolgus monkeys. The results are presented in the table below:

Table I Effects of Interferon-b 1a on Leukocyte 2',5'-OAS Activity

Species Tested	Amount of 2', 5'-OAS Activity[a]		
	Control	50 IU/ml rhIFN-b	500 IU/ml rhIFN-b
CD1 mouse	121	84	n.d. [c]
Sprague Dawley rat	148	133	109
NZW rabbit	n.q.[b]	n.q.	n.q.
Beagle dog	1328	2515	2251
Marmoset	n.q.	n.q.	n.q.
Cynomolgus monkey	n.q.	n.q.	n.q.
Human	179	84	558

[a] Data have been normalized, and are presented as pmol of 2',5'-oligoadenylate-5'-triphosphate produced/10^4 cells/hour.
[b] n.q. = not quantifiable; below lower limit of detection of assay (< 30 pmol/10^4 cells)
[c] n.d. = not done

Comment: The sponsor states that the apparent high degree of 2'5'-OAS activity noted in the beagle dog was due to contamination by erythrocytes and subsequent hemolysis.

By contrast, slight dose-related, although statistically not significant increases in neopterin levels in culture supernatant fluid were detected in rhIFN-β treated PBL from rats, mice, beagle dogs and humans. Again, no stimulation of neopterin synthesis was detected in PBL obtained from rabbits, marmosets, or cynomolgus monkeys. The data are presented in the table below:

Table II Effects of Interferon-b 1a on Leukocyte Neopterin Secretion

Species Tested	Amount of Neopterin Activity[a]		
	Control	50 IU/ml rhIFN-b	500 IU/ml rhIFN-b
CD1 mouse	0.18	0.17	0.22
Sprague Dawley rat	0.11	0.12	0.16
NZW rabbit	0.15	0.13	0.12
Beagle dog	0.10	0.13	0.17
Marmoset	0.21	0.22	0.20
Cynomolgus monkey	0.18	0.19	0.15
Human	0.21	0.15	0.26

[a] data are presented as ng/ml neopterin in supernatant fluid obtained after XXXXXXXXXX culture

Activity of β2-microglobulin either prior to or after stimulation with rhIFN-β was detectable only in cells from human donors. Supernatant fluid from human PBL incubated with vehicle control had a concentration of 136 ng/ml β2-MG, which was not

increased after XXXXXXXXXX incubation with 50 U/ml rhIFN-β (131 ng/ml β2-MG). Only a slight increase to 149 ng/ml was detected in supernatant fluid from human PBL cultured for XXXXXXXXXX with 500 IU/ml rhIFN-β. β2-Microglobulin levels in supernatant fluids from PBL from all other species were below the limits of quantitation of the assay (< 25 ng/ml) at all dose levels.

Secretion of the pro-inflammatory cytokines TNF-α and interferon-γ into culture supernatant fluids was measured by ELISA, after XXXXXXXXXX of incubation of PBL from the different species with either vehicle control, 50, or 500 IU/ml rhIFN-β. Assays used substrate and detection antibodies specific for the human cytokines for samples from the marmoset, cynomolgus monkey, and human cells, while an ELISA kit specific for murine cytokines was used to analyze samples from the rats and mice. Supernatant samples from control and rhIFN-β-treated rabbit and dog cells were assayed using both the human and the murine-specific test kits.

Cytokine activity was not appreciably increased in PBL isolated from human donors, or any of the test animal species after incubation with rhIFN-β. For the most part, cytokine activity was only detectable in cells from human donors and in the case of TNF-α, in cynomolgus monkeys to a much lesser degree. The data are presented in the table below for those two species:

Table III Effects of Interferon-b 1a on Leukocyte Cytokine Secretion

Species Tested	**Amount of Cytokine Activity[a]**		
Human	**Control**	**50 IU/ml rhIFN-b**	**500 IU/ml rhIFN-b**
TNF-α	1464	1253	487
Interferon-γ	120	100	n.q.[b]
TGF-β	2.5	2.4	2.8
Cynomolgus monkey			
TNF-α	140	103	35
Interferon-γ	n.d.[c]	n.d.	n.d.
TGF-β	4.0	3.6	3.3

[a] cytokine activity is expressed as ng/ml in culture supernatants obtained after XXXXXXXXXX exposure
[b] n.q. = below the lower limit of detection of the assay (< 100 pg/ml)
[c] n.d. = not detectable (not distinguishable from 0)

The ability of IFN-β to stimulate synthesis and release of these pharmacodynamic markers was also evaluated in serum and PBL samples obtained from cynomolgus monkeys undergoing repeat-dose toxicity studies, as part of the characterization of the activity of the product *in vivo.* Cynomolgus monkeys were treated for 28 d by s/c injection with 0, 1, 3, or 10 MIU/kg/d rhIFN-β, and peripheral blood samples were obtained for analysis of these markers at 2, 6, and 24 h after treatment on d 1 and d 22. There was no clear increase in serum level of TGF-β as compared to control at any of the

sampling times. However, when values were compared within the dose groups, time-related increases in TGF-β were observed in monkeys treated with either 1 or 3 MU/kg/d at the d 1 time point only. Maximal TGF-β levels in the control and high-dose animals were seen at 2 h after treatment, and were not significantly different between the two groups. At d 22, the majority of samples were not analyzed due to insufficient serum obtained; only samples from animals in the high dose group were analyzed, and there were no differences noted between the three time points assayed.

Enzyme activities of 2', 5'-OAS were below the limit of detection in PBL samples obtained form monkeys in all dose groups at all time points on study (data not shown). Serum neopterin levels were significantly increased from both pre-study baseline levels and from vehicle control-treated monkeys in all three IFN-β-treated groups in a dose-related manner at 24 h after dosing on d 1 only, with a trend towards a dose-related increase at 6 h after dosing. At d 22 of dosing, the neopterin response to IFN-β was blunted as compared to the values obtained at d 1, although evidence of a dose-related effect was suggested, especially at the 6 h time point. The data are presented in the table, below:

Table IV Effects of Interferon-b 1a on In Vivo Leukocyte Neopterin Secretion

rhIFN-β (MIU/kg/d, s/c)	Serum Neopterin Concentration (ng/ml)			
	Pre-Study	Sampling Time (h after injection)	Treatment Period	
			Day 1	Day 22
0	1.0	2	0.9	0.7
	0.9	6	0.7	0.8
	0.8	24	0.8	0.8
1.0	0.6	2	0.5	0.9
	0.8	6	1.0	1.3
	0.9	24	5.9	1.9
3.0	0.7	2	0.6	1.1
	1.0	6	1.4	3.3
	0.9	24	7.2	1.4
10.0	1.7	2	0.8	7.1
	0.8	6	1.7	9.8
	1.0	24	25.6	1.6

In a second series of experiments, 1 male and one female cynomolgus monkey each received a single, s/c injection of 1 or 10 MIU/kg IFN-β. Peripheral blood leukocytes were obtained by XXXXXXXXXX immediately prior to, and at 6 and 24 h after dosing and analyzed for release of pharmacodynamic markers of interferon activity in culture fluid samples 24 h later.

There was no clear dose- or time-related increase in the supernatant levels of TGF-β at any point after treatment with IFN-β. A two-fold increase in culture supernatant levels of TNF-α, as compared to pre-study level was observed in samples from both male

monkeys at 24 h after treatment with IFN-β; levels were 330 and 758 pg/ml at baseline and 24 h after treatment, respectively, for the low dose male, and 108 and 219 pg/ml for the high dose animal at these same two time points. Both the baseline and 24 h TNF-α levels obtained for the animal treated with 1 MIU/kg were approximately 3-fold greater than those obtained from the cells from the high-dose male at these same time points. Tumor necrosis factor-α levels were not quantifiable pre-dose or at 6 h after dosing in the high-dose female monkey, but had detectable levels of 123 pg/ml secreted by the cells obtained 24 h after dosing *in vivo.* No increase in culture supernatant TNF-α from baseline level was observed for the female monkey in the low dose group (data not shown).

Levels of 2', 5'-OAS in PBL were analyzed at the same time points after dosing. In all cases, increases in enzyme activity from baseline were observed after dosing with IFN-b, with peak effect observed in ¾ monkeys at 6 h post-dose, and at 24 h after injection in the fourth animal. The data are presented in the table, below:

Table V – Effects of Interferon-b 1a on 2', 5'-OAS Levels After In Vivo Dosing

Animal Number	IFN-b Dose (MIU/kg)	Sampling Time (hr)	2', 5'-OAS Level (pmol/10^4 cells/hr)
1 M	1	pre-dose	11
		6	84
		24	111
1 F	1	pre-dose	8
		6	99
		24	43
10 M	10	pre-dose	9
		6	155
		24	127
10 F	10	pre-dose	14
		6	192
		24	117

So, in summary, the data demonstrate that IFN-β can stimulate the *de novo* synthesis and secretion of neopterin, and induce significant levels of 2', 5'-OAS enzyme activity in PBL from non-human primates and man, but not from other species such as mouse, rat, rabbit, or dog. There did not appear to be a dose-relationship in the amount of 2', 5'-OAS induced by the treatment; however, only 2 monkeys were treated at each dose level, and whether these effects were due to individual animal variability or to saturation of the receptor-mediated signally is not possible to determine. These data were used to select the species for further toxicologic and pharmacokinetic evaluation of IFN-β.

Pharmacokinetics Study Summary:

1. Recombinant human interferon-β. *In vitro* determination of the partitioning between plasma and red blood cells. Study #XXXXXXXXXX. Heparinized blood from rats, monkeys, and humans; IFN-β, lot #XXXXXXXXXX; GLP; 6/28 – 9/10/95; XXXXXXXXXX.

2. Recombinant human interferon-β. Single dose pharmacokinetic study in rats by intravenous, intramuscular, and subcutaneous route. Study #XXXXXXXXXX. Sprague-Dawley Crl:CD(SD) BR rats, weight range 109–196 g; 30/sex/group; 1 MIU/kg IFN-β, lot #XXXXXXXXXX, i/v, i/m, or s/c; terminal sacrifice 3/sex/time point for P/K blood levels; GLP; 6/7 – 6/27/88; XXXXXXXXXX.

3. Recombinant human interferon-β. Single dose pharmacokinetic study in monkeys by intravenous, intramuscular, and subcutaneous route. Study #XXXXXXXXXX. *Macaca fasicularis*, weight range 3.3-4.0 kg (males), 2.5-2.9 kg (females); 3/sex; 1 MIU (3.7 μg)/kg IFN-β, lot #XXXXXXXXXX, i/v, i/m, or s/c; three-way cross-over design with 6-9 d washout period; GLP; 4/7 – 4/19/88; XXXXXXXXXX**.**

4. r-hIFN-β. Pharmacokinetic and pharmacodynamic study after single intravenous and subcutaneous administration in monkeys. Study #XXXXXXXXXX. . *Macaca fasicularis*, weight range 2.64–3.65 kg (males), 2.40-23.07 kg (females); 3/sex/dose level; 1, 3, 10 MIU/kg IFN-β, lot #XXXXXXXXXX, i/v or s/c; sequential design with 7 d washout period; GLP; 2/28 – 7/11/96; XXXXXXXXXX**.**

Pharmacokinetics Study Review:

Study #XXXXXXXXXX. *Recombinant, human interferon-b. In vitro determination of the partitioning between plasma and red blood cells.*

To evaluate the blood:plasma ratio of IFN-β and its subsequent effects on the pharmacokinetics and pharmacodynamics on interferon *in vivo*, an *in vitro* study was conducted to determine the partitioning of radiolabeled (^{125}I-) IFN-β in red blood cells and plasma.

Radiolabeled, ^{125}I-IFN-β was obtained by direct labeling of the protein, to achieve a specific activity at the time of the assay of XXXXXXXXXX. This material was then incubated with 2 ml aliquots of heparinized human, rat, or monkey blood for various time points at XXXXXXXXXX, at 200 IU/sample, or for XXXXXXXXXX at varying temperatures from XXXXXXXXXX. An additional assay to determine the effects of test article concentration on partitioning between erythrocytes and plasma was conducted by incubating red blood cells from the three species in XXXXXXXXXX ^{125}I-IFN-β with additional XXXXXXXXXX IFN-β added to bring the final sample concentrations to 40, 100, 200, 400, or 800 IU IFN-β/sample.

Following incubation with the radiolabeled compound, two aliquots were removed for determination of whole blood radioactivity. The remaining material was centrifuged over XXXXXXXXXX, and the red blood cell and plasma fractions were collected, the red cells lysed by XXXXXXXXXX, and two aliquots per sample of both the plasma and cell lysates evaluated for uptake of the radiolabel by XXXXXXXXXX. The concentration of IFN-β in the plasma fractions was also determined by ELISA, using a commercially available test kit with a lower limit of detection of 5 IU IFN-β/ml.

There was no significant uptake or partitioning of IFN-β into the red cell fractions, as compared to the plasma levels. After XXXXXXXXXX centrifugation and washing, the majority of the radiolabel was in the plasma fraction and in the supernatant fluid from the cell washes. Only 3-4% of the total ^{125}I-radiolabel was associated with the cell lysates, regardless of the species tested.

There were no significant effects of either time or temperature of incubation on red cell uptake of ^{125}I-IFN-β. After 15 min incubation, between 33 and 39% of the total radioactivity was in the plasma fraction. This value increased to approximately 50% by 30 to 60 min, and decreased back to approximately 40% of the total radiolabel in the plasma by 120 min of incubation. The values obtained for the amount of ^{125}I-IFN-β in rat, monkey, or human plasma were approximately the same at all time points, regardless of the source of the blood samples.

After a 30 min incubation at XXXXXXXXXX, approximately 60% of the radiolabel was found in the plasma fraction of blood samples from all three species. Uptake and partitioning into the red cells was not significantly increased as the temperature of incubation increased; between 45 to 50% of the ^{125}I-IFN-β was present in the plasma when the temperatures were raised to XXXXXXXXXX. Addition of non-radiolabeled IFN-β had no effects on plasma fractionation of the radiolabeled material. At levels of up to 100 IU, between 45 and 50% of the radioactivity was in the plasma fraction and was only slightly increased to 54% and 58% when 200 IU and 400 IU of XXXXXXXXXX IFN-β, respectively, were added to the incubation mixture.

Due to interference by red cells and other matrix factors, the ELISA method was not suitable for quantitating IFN-β in whole blood or cell lysates. Analysis of plasma samples and of serial dilutions of the ^{125}I-labeled IFN-β revealed a complete loss of the immunological recognition of the material by the anti-IFN-β capture antibody (*i.e.* a sample "spiked" with XXXXXXXXXX ^{125}I-IFN-β gave a reading in the ELISA assay that was below the lower limit of detection of 5 IU/ml). Biologic activity in the interferon CPE assay was also completely lost following radiolabeling of the compound.

In summary, the partitioning studies described here reveal that after in vitro exposure of human, monkey, or rat blood to radiolabeled IFN-β, approximately 50% of the material is retained in the plasma. There were no significant effects of either time or temperature of incubation on partitioning of the ^{125}I-IFN-β, and only minor shifts in the plasma levels

were observed when increasing concentrations of unlabelled IFN-β were added to the mixture. The remaining material was not found to be associated with red blood cell lysates, but in the supernatant fractions of the wash and centrifugation buffers, suggesting that it is only loosely associated with the cellular fraction. These data suggest that the binding of IFN-β to red cells is mainly non-specific, and that the material is not actively taken up into erythrocytes. Additionally, the ELISA and CPE studies showed that the addition of the radiolabel destroyed both the biologic and immunologic activity of the protein. Therefore, the significance of these data in respect to the *in vivo* distribution of IFN-β is unknown.

Study #XXXXXXXXXX. *Recombinant human interferon-b. Single dose pharmacokinetic study in rats by intravenous, intramuscular, and subcutaneous route.*

The pharmacokinetics of IFN-β were determined in rats after a single i/v, i/m, or s/c injection of 1 MIU/kg. Three animals per sex were sacrificed at each time point after injection, and serum samples obtained. Interferon-β levels in serum were determined by CPE assay, using human WISH cells infected with VSV as previously described, and pharmacokinetic profiles were calculated for each route of administration, using non-parametric methods.

After i/v injection, the mean serum IFN-β concentration *vs*. time profile declined in a bi-exponential fashion, with a t½α of 0.3 h for distribution, and a t½β of 1.6 hours. The volume of distribution was approximately equivalent to total body water, suggesting extensive distribution of the biologic. Absorption of IFN-b after i/m injection was rapid, with a peak concentration reached at 1 h. At 15 min after injection, the mean serum level of INF-β was approximately 60% of that reached at the 1 h time point, and declined in a bi-exponential fashion afterwards. Bioavailability of IFN-β after i/m injection was approximately 21%, as determined by the ratio of the AUCs between i/v and i/m injection.

Following s/c injection of IFN-β, a longer absorption phase and a lower level of peak concentration was achieved, as compared to i/m injection. A plateau in serum concentration of IFN-β was achieved between 1 and 4 h after injection, with concentrations ranging between 70 and 95 IU/ml, and declining thereafter. By 24 h after injection (the last time point sampled), detectable IFN-β activity was still present in the serum, at a mean level of 29 IU/ml. Bioavailability of IFN-β after s/c injection was approximately 16%, as determined by the ratio of the AUCs between i/v and s/c injection. A summary of the results is provided in the table, below:

Table VI - Pharmacokinetic Profiles of IFN-b in Sprague-Dawley Rats

P/K Parameter	IV injected	IM Injected	SQ Injected
Cmax (IU/ml)	8486	264	95
Tmax (h)	0.08	1	3
$t_{½_b}$ (h)	1.58	-n.d.-	-n.d.-
$AUC_{(0\text{-}last)}$ (h*IU/ml)	6228	1447	1064
$AUC_{(0\text{-}\infty)}$ (h*IU/ml)	6785	-n.d.-	-n.d.-
MRT_{last} (h)	1.01	7.49	9.21
V_c (ml/kg)	101	-n.d.-	-n.d.-
Cl (ml/kg/h)	147	-n.d.-	-n.d.-
Bioavail (%)	-n.a.-[b]	21	16

[a] n.d. = not determined
[b] n.a. = not applicable

In summary, IFN-β was well absorbed after i/m injection, and more slowly absorbed but at a similar bioavailability after s/c injection. Peak concentrations of serum IFN-β were significantly lower after i/m or s/c injection than after i/v administration of the same (1 MIU/kg) dose.

Study #XXXXXXXXXXX. *Recombinant human interferon-b. Single dose pharmacokinetic study in monkeys by intravenous, intramuscular, and subcutaneous route.*

The pharmacokinetics of IFN-β were determined in cynomolgus monkeys after a single i/v, i/m, or s/c injection of 1 MIU/kg. Three animals per sex were treated in a three-way, cross-over study design, with a 6-8 day washout period between treatments. Animals were fasted overnight prior to each study treatment, and samples of blood were obtained at various time points following injection. Interferon-β levels in serum were determined by CPE assay, using human WISH cells infected with VSV as previously described, and pharmacokinetic profiles were calculated for each route of administration, using non-parametric methods.

All monkeys had detectable serum IFN-β activity prior to treatment with the test article, with a mean value of 64 $\pm$ 44 IU/ml at baseline. After i/v injection, peak concentrations were observed in 5/6 monkeys at 5 min, with a mean value of 18409 $\pm$ 7886 IU/ml. One monkey (animal #8F) had a much lower serum IFN-β value at 5 min after injection (3693 IU/ML), as compared to the mean value for the other five monkeys, but was not excluded from the calculation. Following peak levels, the mean serum IFN-β concentration *vs.*

time profile declined in a bi-exponential fashion, with a t½α of 0.11 to 0.26 h for distribution, and a t½β of 1.1 to 1.7 hours. The volume of distribution was approximately equivalent to plasma volume, suggesting a less extensive distribution of the biologic in non-human primates than in rodents (see Study #XXXXXXXXXX, above).

After i/m injection of IFN-β, several peaks were observed in all of the monkeys, suggestive of a "flip-flop" pharmacokinetic profile. Although no pharmacokinetic model could be used to describe the serum profiles, absolute peak concentrations were observed between 1 and 8 h after injection. The mean absolute bioavailability of IFN-β after i/m injection was approximately 41%, as determined by the ratio of the AUCs between i/v and i/m injection at the 24 h time point.

Following s/c injection, the serum IFN-β profiles were similar to those seen after i/m administration, with each animal exhibiting more than one peak and a "flip-flop" profile. Absolute peak IFN-β levels were achieved between 3 and 8 h after injection, with concentrations ranging between 45 and 718 IU/ml, and declining thereafter. By 24 h after injection (the last time point sampled), detectable IFN-β activity was still present in the serum, at a mean level of 29 IU/ml. Bioavailability of IFN-β after s/c injection was approximately 38%, as determined by the ratio of the AUCs at the last time point, between i/v and s/c injection. A summary of the results is provided in the table, below:

Table VII - Pharmacokinetic Profiles of IFN-b in Cynomolgus Monkeys

P/K Parameter	IV injected	IM Injected	SQ Injected
Cmax (IU/ml)	18409	617	389
Tmax (h)	0.08	3	4.3
$t_{½_b}$ (h)	1.43	-n.d.-[a]	-n.d.-
$AUC_{(0\text{-}last)}$ (h*IU/ml)	16743	6908	6397
$AUC_{(0\text{-}\infty)}$ (h*IU/ml)	18716	-n.d.-	-n.d.-
MRT_{last} (h)	1.41	-n.d.-	-n.d.-
V_c (ml/kg)	35.2	-n.d.-	-n.d.-
Cl (ml/kg/h)	54.5	-n.d.-	-n.d.-
Bioavail (%)	-n.a.-[b]	41	38

[a] n.d. = not determined
[b] n.a. = not applicable

In summary, IFN-β after i/m or s/c injection in cynomolgus monkeys exhibited "flip-flop" pharmacokinetic profiles, but yielded similar bioavailability after injection by either

route. Peak concentrations of serum IFN-β were significantly lower after i/m or s/c injection than after i/v administration of the same (1 MIU/kg) dose. Significant inter-animals variability was present in each treatment group, which may account for some of the very broad CVs obtained for each pharmacokinetic parameter (data not shown).

Study #XXXXXXXXXX. r-hIFN-b. *Pharmacokinetic and pharmacodynamic study after single intravenous and subcutaneous administration in monkeys.*

A comparison of the dose-related effects of IFN-β on the pharmacokinetic profiles and induction of pharmacodynamic markers was conducted in cynomolgus monkeys after a single dose of the product. Three monkeys per sex received a single, i/v dose of either 1, 3, or 10 MIU/kg IFN-β (3,7, 11, or 37 µg/kg, respectively), and blood collected at various time points for analysis of serum IFN-β and neopterin levels, and of 2', 5'-OAS levels in PBL. Following a one-week washout period, animals were treated with the same dose of IFN-β by s/c injection, blood samples collected, and the same parameters analyzed for comparison. Serum levels of IFN-β were analyzed by ELISA using a commercially available kit validated for use with monkey serum. Neopterin and 2', 5'-OAS were analyzed by RIA techniques using commercially available kits. The lower limit of detection for each of these assays was 5 IU/ml for the IFN-β ELISA, 30 pmol ATP converted/dl/h for the 2', 5'-OAS enzyme RIA, and 0.5 ng/ml for the neopterin RIA.

Pharmacokinetic profiles for IFN-β demonstrated non-linear relationships of peak serum level to initial dose administered, after either i/v or s/c injection. Following a single i/v injection, concentrations of IFN-β in the serum and the calculated AUC values were 2 to 4 times higher than predicted, given the doses of the drug administered (r^2 = 0.999 for C_{max}, 0.991 for AUC). As the dose of IFN-β was increased, the steady state volume of distribution and the clearance decreased, suggesting that IFN-β distribution and elimination involved saturable mechanisms (please see Table VIII, below). There was a wide degree of variability between the animals, and in general, female monkeys appeared to have higher initial serum levels and faster clearance of IFN-β than did the males, although the differences were not statistically significant (data not shown).

After s/c injection, the time to peak serum IFN-β levels was variable between individual monkeys, but was approximately 3 to 6 h. Again, both AUC values and peak concentrations were greater than predicted, but the relationship was approximately linear (r^2 = 0.997 for Cmax, 0.983 for AUC). Mean residence times were not significantly different, regardless of the dose of IFN-β administered; however, the elimination half-life decreased with increasing dose of IFN-β. Clearance and steady-state volume of distribution were not determined for this route of administration.

Comment: Independent evaluation of the individual animal data by this reviewer did not demonstrate the same sort of "flip-flop" pharmacokinetic profiles, with multiple peak concentrations that were obtained in the previous study after either i/m or s/c injection of 1 MIU/kg IFN-β. However, the mean values presented by the sponsor and the sponsor's

interpretation of the data suggest that this event did occur. The reason(s) for the discrepancy is not clear at the present time.
Because of the non-linear relationship observed between the dose and resulting serum levels of IFN-β, absolute bioavailability of the product after s/c injection could not be determined. Estimations of the fraction of IFN-β available were calculated by the ratio of AUCs obtained for each dose at the s/c versus i/v routes of administration, and ranged between 12 and 25%. For ease of presentation, values were re-calculated for each dose group by each route of administration, with male and female animal data together. The data are presented in the tables as mean value ± S.D, below:

Table VIII - Pharmacokinetic Profiles of IFN-b in Cynomolgus Monkeys

A. IV Administration

P/K Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (IU/ml)	6360 ± 1728	33600 ± 8913	224200 ± 88480
Tmax (h)	0.08	0.08	0.08
$t½_{elim}$ (h)	3.0 ± 1.8	4.8 ± 2.8	5.8 ± 1.4
$AUC_{(0-last)}$ (h*IU/ml)	3116 ± 955	23213 ± 3159	131428 ± 17648
$AUC_{(0-\infty)}$ (h*IU/ml)	3144 ± 959	23275 ± 3163	131509 ± 17642
$MRT_{(0-\infty)}$ (h)	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
V_{dss} (ml/kg)	260 ± 101	101 ± 31	64 ± 20
Cl (ml/kg/h)	348 ± 122	131 ± 20	77 ± 11

B. SQ Administration

P/K Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (IU/ml)	48 ± 16	335 ± 169	1740 ± 605
Tmax (h)	3.8 ± 2.5	3.0 ± 0	3.7 ± 2.7
$t½_{elim}$ (h)	9.0 ± 3.8	7.3 ± 2.0	5.9 ± 1.0
$AUC_{(0-last)}$ (h*IU/ml)	600 ± 327	2681 ± 518	24454 ± 5272
$AUC_{(0-\infty)}$ (h*IU/ml)	750 ± 422	2925 ± 575	24980 ± 5188
$MRT_{(0-\infty)}$ (h)	13.8 ± 5.1	10.3 ± 2.3	11.3 ± 0.7
Bioavail (%)	24.8 ± 12.4	12.8 ± 2.1	19.3 ± 4.9

Serum neopterin and PBL 2', 5'-OAS levels were also increased after IFN-β treatment, by either route of administration. All animals had detectable levels of neopterin prior to administration of the biologic; these values were subtracted from the individual animal's values at each time point for calculation of the mean values. Peak neopterin levels were observed approximately 24 h after dosing with IFN-β by the i/v route and between 48 and 72 h after treatment by s/c injection. There was a slight relationship in the amount of neopterin induced to the dose of IFN-β administered; however, there was no difference in the magnitude of neopterin induction after i/v as compared to s/c injection. Neopterin levels remained elevated over baseline in all animals at the 96 h time point, which was the last point measured. The data are presented in the table, below:

Table IX – Effects of IFN-b treatment on Serum Neopterin Levels in Cynomolgus Monkeys

A. IV Administration

P/D Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (ng/ml)	8.2 ± 2.1	10.2 ± 2.1	17.1 ± 5.4
Tmax (h)	22 ± 4.9	28 ± 9.8	36 ± 13.1
$AUC_{(0\text{-}last)}$ (h*ng/ml)	419 ± 160	634 ± 119	1112 ± 334

B. SQ Administration

P/D Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (ng/ml)	6.9 ± 2.7	9.3 ± 1.9	18.6 ± 5.9
Tmax (h)	52 ± 9.8	60 ± 20	36 ± 13.1
$AUC_{(0\text{-}last)}$ (h*ng/ml)	444 ± 181	584 ± 166	1221 ± 416

Levels of 2', 5'-OAS were also increased following either i/v or s/c injection with IFN-β. The majority of animals did have quantifiable levels of enzyme activity at baseline, which was subtracted from each individual value for the purposes of data calculation. There was no clear dose-relationship towards increased enzyme activity (C_{max} or AUC) with increasing dose of the biologic after either i/v or s/c injection. The data are presented in the table, below:

Table X – Effects of IFN-b treatment on PBL 2', 5'-OAS Levels in Cynomolgus Monkeys

A. IV Administration

P/D Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (pmol/dl/h)	129 ± 83.5[a]	209 ± 86.7	201 ± 5.4
Tmax (h)	20 ± 16.8	24 ± 13.1	34 ± 15.9
$AUC_{(0\text{-}last)}$ (h*pmol/dl/h)	6313 ± 4161	11323 ± 5933	11221 ± 5741

B. SQ Administration

P/D Parameter	1 MIU/kg	3 MIU/kg	10 MIU/kg
Cmax (pmol/dl)	95 ± 62.1	119 ± 57.1	237 ± 96.5
Tmax (h)	22 ± 4.9	31 ± 24.7	58 ± 35.9
$AUC_{(0\text{-}last)}$ (h*pmol/dl/h)	5171 ± 3886	5246 ± 3299	9873 ± 6263

In summary, administration of IFN-β by either i/v or s/c injection to cynomolgus monkeys was associated with increased serum levels of the biologic, which were greater than predicted (both C_{max} and AUC) for the doses employed. The material was biologically active in this species, as evidenced by elevations in serum neopterin and PBL 2', 5'-OAS levels; however, these increases were not completely linear in proportion to dose of IFN-β administered. The two pharmacodynamic markers appear to be indicative of biologic activity of this agent, and may predict clinical activity in the humans, as well.

PRECLINICAL TOXICOLOGY:

Mutagenicity Study Summary:

1. Study of the capacity of the test article Interferon Beta, recombinant to induce gene mutations in strains of Salmonella typhimurium. Study #XXXXXXXXXX. IFN-β (lot #XXXXXXXXXX, experiment #1, lot #XXXXXXXXXX, experiment #2, specific activity not provided); 0.01, 0.05, 0.25, 0.5 MU/plate, with and without XXXXXXXXXX GLP; 3/1 – 3/7/88; XXXXXXXXXX.

2. Study of the capacity of the test article Interferon Beta, recombinant to induce "unscheduled DNA synthesis" in cultured HeLa cells. Study #XXXXXXXXXX. IFN-β (lot #XXXXXXXXXX, both experiment #1 and experiment #2, specific activity not provided); XXXXXXXXXX

3. Study of the capacity of the test article Interferon Beta, recombinant to induce chromosome aberrations in human lymphocytes cultured *in vitro*. Study #XXXXXXXXXX. 0.9% sterile saline, IFN-β (lot #XXXXXXXXXX, specific activity not provided); 0.004, 0.02, 0.04, 0.2 MU/ml, with and without XXXXXXXXXX.

4. Micronucleus induction in bone marrow cells of mice treated with the test article Interferon Beta, recombinant by intravenous route. Study #XXXXXXXXXX. Crl:CD-1 (ICR) BR mice; 15/sex/group, weight range 22-31 g; 0.9% sterile saline, 20 MIU/kg IFN-β (lot #XXXXXXXXX, specific activity not provided), i/v; mitomycin C, 8 mg/kg, i/p; GLP; 4/26 – 6/10/88; XXXXXXXXXX.

5. *In vivo* study of chromosome aberration in the Chinese hamster bone marrow induced by the test article Interferon Beta, recombinant administered by intraperitoneal route. Study #XXXXXXXXXX. Crl:CD-1 (ICR) BR mice; 2/sex/group, weight range 24-35 g; 0.9% sterile saline, 5, 10, 20 MIU/kg IFN-β (lot #XXXXXXXXXX, specific activity not provided), i/p; mitomycin C, 12 mg/kg, i/p; GLP; 3/1 – 6/23/88; XXXXXXXXXX.

Mutagenicity Study Review:

Study #XXXXXXXXXX. ***Study of the capacity of the test article Interferon Beta, recombinant to induce gene mutations in strains of Salmonella typhimurium.***

Cultures of XXXXXXXXXX strains of *Salmonella typhimurium* were incubated in triplicate at XXXXXXXXXX with IFN-β at multiple concentrations ranging from XXXXXXXXXX. The IFN-β preparation was diluted in vehicle (sterile 0.9% saline) in the presence and absence of metabolic activation by XXXXXXXXXX. The highest concentration of IFN-β tested was the highest level that could feasibly be utilized in this system, based on reconstitution vial concentrations. Positive control chemicals used were 9-aminoacridine (XXXXXXXXXX), 2-aminofluorine (XXXXXXXXXX), hydrazine sulfate (XXXXXXXXXX), and doxorubicin (XXXXXXXXXX). Sterile Water for Injection, USP and 0.9% sterile saline solution (vehicle control) were employed as negative controls.

No range-finding assay was performed for IFN-β; the dose-response was incorporated as part of the initial study and encompassed a three log range, and the study was repeated with similar results at the same test concentrations of IFN-β (see above). Interferon-β exhibited no toxicity to the test strains doses as high as XXXXXXXXXX. All of the test strains treated with IFN-β exhibited mean reversion frequencies (number of histidine$^+$ revertant colonies above the control incidence) similar to both vehicle and solvent controls, with or without XXXXXXXXXX metabolic activation, and there was no evidence of dose-related effects up to the highest concentration evaluated. Each positive control produced a marked mutagenic response in the appropriate strain.

In summary, IFN-β exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, using the standard Ames microbial mutagenicity plate incorporation tests.

Study #XXXXXXXXXX. *Study of the capacity of the test article Interferon Beta, recombinant to induce "unscheduled DNA synthesis" in cultured HeLa cells.*

The ability of IFN-β to induce repairable damage to DNA was evaluated in human HeLa (cervical carcinoma) cells *in vitro*. The process of DNA repair by excision and replication occurs by the removal of the damaged DNA strand sections, followed by synthesis of the corresponding, correct nucleotide sequence. Radiolabeled nucleotide precursors (XXXXXXXXXX) are added to the cells in the presence of the test article and hydroxyurea (to suppress normal DNA replication). Agents which increase the rate of "unscheduled" DNA synthesis will result in an increase in the amount of XXXXXXXXXX incorporated into the cellular DNA, which may be quantitated by β-scintillation counting after extraction from the cells.

For each experiment, XXXXXXXXXX HeLa cells were plated in 24-well culture dishes and allowed to expand for XXXXXXXXXX. Three wells were used for each concentration of test article, and incubated for XXXXXXXXXX in the presence and absence of XXXXXXXXXX hydroxyurea. At this point, the cultures were washed, and exposed to various concentrations of the test article, or the positive and negative control agents, in the presence and absence of XXXXXXXXXX activation. Positive control agents included XXXXXXXXXX final concentration methyl methanesulfonate (for the test without metabolic activation), and XXXXXXXXXX cyclophosphamide (as positive control for the test with metabolic activation by XXXXXXXXXX fraction). After XXXXXXXXXX of exposure, the cells were washed, treated for XXXXXXXXXX with fresh medium with or without XXXXXXXXXX hydroxyurea, then pulsed with XXXXXXXXXX. Following incubation with the radiolabel, the cells were washed, precipitated with XXXXXXXXXX, and an aliquot of trypsinized cells evaluated for uptake of the radiolabel by liquid scintillation spectrophotometry.

In two separate experiments, incubation of HeLa cells in the presence of up to 0.5 MIU/plate IFN-β, either with or without metabolic activation did not result in an increase in XXXXXXXXXX uptake and incorporation over the level observed for samples treated with the negative (saline) control. This effect was noted regardless of whether the cells were incubated in the presence or in the absence of hydroxyurea. The ratio of toxicity of the test:control articles (T:C ratio) for IFN-β in both experiments was approximately 1, at all concentrations tested. By contrast, XXXXXXXXXX incorporation was increased by approximately 2 to 3-fold over saline control levels when the cells were exposed to the appropriate positive controls and hydroxyurea. The T:C ratios for both positive control compounds were between 0.67 and 0.83, indicating that cell cytotoxicity was also induced by exposure to the positive controls.

In summary, exposure of cultured HeLa cells to IFN-β at concentrations of up to 0.5 MIU/ml had no significant effects on cell cytotoxicity or DNA damage, as determined by incorporation of XXXXXXXXXX. Therefore, the test article is not considered to induce unscheduled DNA synthesis, as defined by the conditions of this assay.

Study #XXXXXXXXXX. ***Study of the capacity of the test article Interferon Beta, recombinant to induce chromosome aberrations in human lymphocytes cultured in vitro.***

The clastogenic potential of interferon-β was assessed in an *in vitro* assay using mitogen-stimulated, primary human peripheral blood lymphocytes in the presence and absence of XXXXXXXXXX microsomes as an exogenous metabolic activation system. Phytohemagglutinin-stimulated cells obtained from one male human donor were treated with IFN-β at concentrations ranging from XXXXXXXXXX, in the presence or absence of XXXXXXXXXX microsomes. The highest concentration of IFN-β tested was the highest level that could feasibly be utilized in this system. Sterile, 0.9% saline solution was used as a negative control. Mitomycin C XXXXXXXXXX cyclophosphamide were used as positive control agents in the non-activated and activated phases of the study, respectively.

Following exposure to the test articles, the cells were washed, resuspended in fresh culture medium, and incubated for an additional XXXXXXXXXX. The following day, the lymphocytes were harvested and chromosomal specimens prepared after a XXXXXXXXXX exposure to colchicine. Microscopic cytogenetic evaluations were performed on blind coded slides from each sample, with 100 metaphases scored for chromosomal aberrations from each of two replicate cultures. Relative mitotic indices were not determined.

The percentage of cells with chromosomal aberrations ranged from 0 to 2% for any of the IFN-β concentrations tested, either with or without XXXXXXXXXX metabolic activation. Sterile saline controls had total aberrant cell indices of 2%, regardless of whether XXXXXXXXXX fraction was present. Aberrations included gaps, breakages, fragmentations, and symmetric or asymmetric interchanges, and occurred at approximately equal incidence in the saline control and in all IFN-β-treated groups, with or without metabolic activation. The positive controls produced the expected clastogenic response (100% of the cells examined with aberrancies). The total percentage of aberrant cells in both of the positive control groups exceeded the number of metaphases examined, indicating more than one area of chromosomal damage in several cells (106% for mitomycin C in the non-activated system and 162% aberrant cells with cyclophosphamide in the activated system). These results are in the range of values expected for mitomycin C and cyclophosphamide under the conditions of this assay, confirming the sensitivity of the test system.

In summary, IFN-β showed no evidence of clastogenic potential in this *in vitro* human peripheral blood lymphocyte chromosomal aberration assay at up to the highest feasible concentration (XXXXXXXXXX) which could be evaluated.

Study #XXXXXXXXXX. ***Micronucleus induction in bone marrow cells of mice treated with the test article Interferon Beta, recombinant by intravenous route.***

The potential for IFN-β to induce chromosomal damage was evaluated *in vivo* using the mouse bone marrow micronucleus assay. This test is used to screen agents that cause chromosomal damage, manifested by acentric chromatids and chromosome fragments, which are retained by the daughter cells during mitosis as secondary nuclei. The presence of these micronuclei in the cell cytoplasm constitutes evidence that the DNA has undergone some type of damage.

Micronuclei are typically evaluated in polychromatic erythrocytes from bone marrow of rodent species, for two reasons. Although micronuclei can be detected in myeloblasts, myelocytes, and erythroblasts, they are not easy to distinguish in cell types with either very little cytoplasm or a large nucleus. However, a few hours after undergoing their final mitosis, rodent (and other) mammalian erythrocytes expel the nucleus, yet the micronuclei are retained. Therefore, the easiest cell type to evaluate for micronucleus formation is the polychromatic erythrocyte. Secondly, rodent spleens are unable to phagocytize and clear micronucleated red blood cells, while dog and other large mammalian species are very effective at removing these cells.

Fifteen mice per sex per group were injected i/v with either sterile 0.9% saline or 20 MIU/kg IFN-β on day 1. Five mice of each sex in each group were sacrificed at 18, 42, and 65 h after treatment by cervical dislocation, and femoral bone marrow smears prepared. An additional five mice/sex were treated with 8 mg/kg mitomycin C, i/p as a positive control, sacrificed at 42 h, and bone marrow smears prepared. Two slides were prepared for each individual animal, fixed, and stained with XXXXXXXXXX solution. The stained slides were coded and evaluated microscopically at 1250X magnification. For each animal, a total of 2000 polychromatic erythrocytes were counted and scored for the number and percentage of micronucleated cells. Additionally, the ratio of polychromatic:normochromatic erythrocytes (P:N ratio) for each slide was determined.

The number of micronuclei present in the control cells ranged from 0/2000 to 4/2000, and from 0/2000 to 3/2000 in cells from the IFN-β-treated mice at all time points after injection. By contrast, at 42 h after mitomycin C treatment, the incidence of polychromatic erythrocytes containing micronuclei ranged from 4/2000 to 18/2000, confirming the sensitivity of the assay system. The mean value for incidence of micronucleated red cells in the mitomycin C group was 13.2 $\pm$ 4.2/2000 cells ($p \leq 0.001$, Chi-square), as compared to 1.2 $\pm$ 0.9/2000 cells and 1.5 $\pm$ 1.0/2000 cells for the saline control and IFN-β treated groups at this time point, respectively. Similarly, there were no significant differences in the P:N ratio between the control and the IFN-β-treated groups at any time point assayed. Although not statistically significant, the P:N ratio was lower

for the mitomycin C-treated group than for either the saline control or the test article (mean values, 0.743 ± 0.1, compared to 1.2 ± 0.1 and 1.1 ± 0.1, respectively).

In summary, these data demonstrate that under the conditions of this assay, IFN-β at a dose of 20 MIU/kg, i/v did not induce any statistically significant changes in the incidence of micronucleated bone marrow cells, suggesting that it is not clastogenic after *in vivo* exposure.

Study #XXXXXXXXXX. ***In vivo study of chromosome aberration in the Chinese hamster bone marrow induced by the test article Interferon Beta, recombinant administered by intraperitoneal route.***

The clastogenic potential of IFN-β was assessed in an *in vivo* assay using bone marrow cells derived from Chinese hamsters after injection with various doses of the test article. Two animals per sex were injected at 0 and 24 h with 0.9% saline (negative control), XXXXXXXXXX mitomycin C (positive control), or 5, 10, or 20 MIU/kg IFN-β, i/p. Six hours after the second injection, all hamsters received a single i/p injection of XXXXXXXXXX colchicine. The hamsters were euthanized one hour later, and bone marrow was recovered from the femurs, washed, and fixed in ice-cold methanol:acetic acid (3:1). Four slides of isolated bone marrow cells were prepared for each animal, stained in XXXXXXXXXX solution, and evaluated microscopically at 1250X magnification for evidence of chromosomal aberrations. Microscopic cytogenetic evaluations were performed on blind coded slides from each sample, with 100 metaphases scored for chromosomal aberrations from each animal. Relative mitotic indices were not determined.

The percentages of cells with chromosomal aberrations ranged from 0 to 5% for any of the IFN-β concentrations tested, and were not significantly different from the control group. Sterile saline control animals had total aberrant cell indices of 1-2%. Aberrations included gaps, breakages, and fragmentations, and occurred at approximately equal incidence in the saline control and in all IFN-β-treated groups. The positive control produced the expected clastogenic response (47 to 95% of the cells examined with aberrancies). All four animals treated with mitomycin C had between 10 and 21% metaphases with more than one aberration. These results are in the range of values expected for mitomycin C under the conditions of this assay, confirming the sensitivity of the test system.

The results of this assay demonstrate that treatment of Chinese hamsters with IFN-β at 5, 10, or 20 MIU/kg is not associated with significant, *in vivo* clastogenic potential, as defined by a lack of chromosomal aberrations in bone marrow samples.

Comment: The studies employed using the *Salmonella typhimurium* bacterial mutagenesis assays (Ames test) are inappropriate for protein biotherapeutic agents. Similarly, the *in vitro* mutagenesis assay using cultured human peripheral blood mononuclear cells and the *in vivo* micronucleus assay in mice and chromosomal

aberration assay in hamsters will not provide information regarding the clastogenic potential of IFN-β. These assays are designed to detect drugs, chemicals, and environmental agents that cause direct damage to DNA molecules. Interferon-β1a is a recombinant, glycosylated protein; the chances of its interacting directly with DNA and causing mutagenic changes are remote, as evidenced by the negative results in the described assays.

Toxicology Study Summary:

1. Single dose toxicity study in rats and mice treated with the test article interferon Beta, recombinant administered by intravenous and intramuscular route. Study #XXXXXXXXXX. Crl:CD-1 (ICR) BR mice, 5/sex/group, 22-25 d old, weight range 13-15 g on receipt; Crl:CD (SD) BR Sprague-Dawley rats, 5/sex/group, 5-6 wks old, weight range, 100-125 g on receipt; vehicle control (sterile, 0.9% saline), 10, 20 MIU/kg IFN-β (lot #XXXXXXXXXX); volume of injection 10 ml/kg by i/v, 2 ml/kg by i/m routes; GLP; 2/15 – 3/4/88; XXXXXXXXXX**.**

2. Acute toxicity study in the cynomolgus (*Macaca fasicularis*) monkey of the test article interferon Beta, recombinant administered by intravenous and intramuscular routes. Study #XXXXXXXXXX. wild-caught monkeys, 1/sex/group, weight range, 2.19 - 2.91 kg; 20 MIU/kg IFN-β (lot #XXXXXXXXXX, concentration 5 MIU/ml); volume of injection 4 ml/kg by either route; GLP; 2/28 – 3/14/89; XXXXXXXXXX**.**

3. 13-week repeated dose toxicity study in Sprague-Dawley Crl:CD (SD) BR rats treated with the test article interferon Beta, recombinant administered by intramuscular route at the dosage of 0, 0.25, 0.5, 1 MIU/kg/day. Study #XXXXXXXXXX. 10/sex/group/time point; 4 wks old, weight range, 75-85 g (male), 60-70 g (female) at time of receipt; placebo control (lot #XXXXXXXXXX), 0.25, 0.5, 1.0 mg/kg/d IFN-β (lots #XXXXXXXXXX, concentration 3 MIU/vial); volume of injection 0.5 ml/kg; interim sacrifice at 4 weeks, final sacrifice at 13 weeks on study (no recovery period); GLP; 2/26 – 5/31/88; XXXXXXXXXX**.**

4. 13-week repeated dose toxicity study in Sprague-Dawley Crl:CD (SD) BR rats treated with the test article Interferon Beta, Recombinant administered by intravenous route at the dosages of 0, 0.25, 0.5, 1 MIU/kg/day. Study #XXXXXXXXXX. 10/sex/group/time point; 4 wks old, weight range, 75-85 g (male), 60-70 g (female) at time of receipt; placebo control (lot #XXXXXXXXXX), 0.25, 0.5, 1.0 mg/kg/d IFN-β (lots #XXXXXXXXXX, concentration 5×10^4 and 1×10^7 IU/ml, respectively); volume of injection 2.0 ml/kg; interim sacrifice at 4 weeks, final sacrifice at 13 weeks on study (no recovery period); GLP; 3/31 – 7/1/88; XXXXXXXXXX**.**

5. 13-Week repeated dose toxicity study in cynomolgus monkeys treated with the test article Interferon Beta, Recombinant administered by intramuscular route at the dosages of 0, 0.25. 0.5, and 1 MIU//kg/day. Study #XXXXXXXXXX. *Macaca fasicularis*, 3/sex/group/time point; wild-caught, weight range 2.23 – 3.62 kg (males), 2.02 – 3.36 kg (females); placebo control (lot #XXXXXXXXXX), 0.25, 0.5, 1.0 MIU/kg/d IFN-β (lots #XXXXXXXXXX, 3 MIU/vial); volume of injection, 0.2 ml/kg; interim sacrifice at 4 weeks, final sacrifice at 13 weeks on study (no recovery period); GLP; 5/18 – 8/19/88; XXXXXXXXXX**.**

6. 13-Week repeated dose toxicity study in cynomolgus monkeys treated with the test article Interferon Beta, Recombinant administered by intravenous route at the dosages of 0, 0.25. 0.5, and 1 MIU/kg/day. Study #XXXXXXXXXX. *Macaca fasicularis*, 3/sex/group/time point; wild-caught, weight range 2.24 – 3.97 kg (males), 2.04 – 3.34 kg (females); placebo control (lot #XXXXXXXXXX), 0.25, 0.5, 1.0 MIU/kg/d IFN-β (lot #XXXXXXXXXX, 3 MIU/vial); volume of injection, 0.5 ml/kg; interim sacrifice at 4 weeks, final sacrifice at 13 weeks on study (no recovery period); GLP; 9/21 – 11/25/88; XXXXXXXXXX**.**

7. 26-week repeated dose toxicity study in Cynomolgus monkeys treated with the test article **REBIF** administered by subcutaneous route at the doses of 0, 3.5, 10.5, and 35 mcg/kg/d. Study #XXXXXXXXXX. *Macaca fasicularis*, approximately 6 - 9 years old, purpose-bred; 4/sex/group/time point, weight range 4.25 – 6.79 kg (males), 2.63 – 4.42 kg (females); vehicle control (0.9% saline, sterile solution, lot #B2), 3.5, 10.5, 35 µg/kg/d IFN-β (lot #XXXXXXXXXX; specific activity 2.83 x 10^8 IU/mg protein); volume of injection, 0.3 ml/kg; GLP; 7/18/95 – 1/19/96; XXXXXXXXXX.

8. Local irritation study in rabbits treated with the test article **REBIF** (finished product) by the intramuscular route. Study #XXXXXXXXXX. New Zealand white, 3 males/group/time point, age range 9 – 10 weeks old, weight range 2.81 – 3.23 kg; 0.5 ml/site; 0.9% saline control, 0.425, 1.7% acetic acid controls; **REBIF** IFN-β, finished lot # XXXXXXXXXX, 12 MIU/ml (specific activity not provided); GLP; 1/24 – 2/7/96; XXXXXXXXXX**.**

9. Evaluation of teratogenic and abortifacient potential of interferon beta recombinant in the cynomolgus monkey. Study #XXXXXXXXXX. *Macaca fasicularis*, 6 pregnant females/group, age range 3 – 15 years old, weight range 2.42 – 3.81 kg at study initiation; placebo control (lot #541/P); 0.2, 0.6, 1.8 MIU/kg/d IFN-β, lot #XXXXXXXXXX, 3.0 MIU/vial (specific activity not provided); administered i/m either GD21-GD89 or GD90-GD150 (term); GLP; XXXXXXXXXX**.**

Toxicology Review:

Study #XXXXXXXXXX. ***Single dose toxicity study in rats and mice treated with the test article interferon Beta, recombinant administered by intravenous and intramuscular route.***

The acute toxicity of IFN-β was determined in outbred rats and mice following a single i/v or i/m injection of the biologic. Animals were treated on d 1 with vehicle control (0.9% sterile saline solution), 10, or 20 MIU/kg IFN-β in saline by i/v injection into the tail vein, or by i/m injection into the left quadriceps femoris muscle. The rats or mice were observed immediately after treatment and at 30 min, 2, 4, and 6 h after the injections for clinical signs of toxicity, then daily for the 14 d duration of the study. Body weights were determined prior to dosing, and on days 3, 8, and 14 immediately prior to euthanization. Following terminal sacrifice, animals underwent full necropsy and evaluation of gross pathologic changes, including organ weights and morphologic changes. Tissue samples from a selected panel of organs from animals in the control and the 20 MIU/kg dose groups were removed and fixed in 10% buffered formalin for evaluation at a later date. Only tissues containing gross pathologic lesions were preserved from the groups of animals treated with 10 MIU/kg IFN-β.

There were no overt signs of clinical toxicity noted in animals from any of the control or IFN-β-treated groups at any time point over the duration of the study. Body weight gains were not appreciably different between the treatment and control animals in either species, or by either route of administration. At necropsy, no gross pathologic lesions were observed in any animals in either the control or treatment groups; therefore, further histologic evaluation was not performed.

In summary, treatment of rats or mice with a single, i/v or i/m injection of REBIF was not associated with any overt signs of toxicity. The NOAEL for IFN-β in these two species is $\geq$ 20 MIU/kg, by either route of exposure.

Comment: Both *in vitro* and *in vivo* pharmacology studies in rats and mice have previously demonstrated that these species are not responsive to recombinant, human interferon-β. Therefore, these data are irrelevant in demonstrating the safety of the biologic for use in clinical studies.

Study #GF-XXXXXXXXXX. ***Acute toxicity study in the cynomolgus (Macaca fasicularis) monkey of the test article interferon Beta, recombinant administered by intravenous and intramuscular routes.***

The acute toxicity of IFN-β was evaluated in male and female cynomolgus monkeys after i/m and i/v injection. One animal per sex received a single injection of IFN-β in saline by either i/v injection into the cephalic vein, or by injection into the anterior and posterior quadriceps muscle. Observations for overt signs of clinical toxicity were performed at 30 min, 1, 2, 4, and 6 h after treatment on d 1, then twice daily for the remainder of the study

duration. Rectal body temperatures were determined on d 1 at these same time points, then at 24 and 48 h post-dosing. Body weights were obtained immediately prior to treatment on d 1, then on days 3, 7, and 15, after an overnight fast. Samples of peripheral blood for determination of hematologic and clinical chemistry profiles were obtained at baseline (days –4 or –3), and at d 15 at the end of the observation period. At study termination, monkeys were euthanized by i/v overdose with sodium pentobarbital solution, and subjected to full necropsy and gross pathologic evaluation. No tissue samples were retained for histologic examination, since no grossly evident, treatment-related lesions were present at time of sacrifice.

There were no deaths on study, and no overt clinical signs of toxicity noted in any of the four monkeys treated with IFN-β. Female monkey #1F (i/v group) showed reduced food intake, however, no loss of body weight was evident over the course of the study. Female monkey #451F had an isolated episode of diarrhea (day on study not specified); this event was determined not to be related to treatment with the test article. Rectal body temperatures were elevated in all four animals by 0.3 to 0.7^{o}C from baseline at 30 min after treatment with IFN-β. Rectal body temperatures continued to increase over the next several observations, reaching peak elevations of 1.2 to 1.8^{o}C by 4 – 6 h after injection, and decreasing to baseline levels by 24 h post-treatment.

Hematologic profiles were not appreciably altered from baseline in any of the four monkeys treated with 20 MIU/kg IFN-β by a single i/v or i/m injection. Slight elevations in AST and ALT were observed in both male monkeys at the end of the observation period; however, these values were still within normal limits for macaque monkeys and the slight elevations were not considered related to the treatment with IFN-β.

At necropsy, no treatment-related findings were evident on gross pathological examination of the IFN-β-treated monkeys. Findings did include focal areas of hemorrhage in the stomach of monkey #6M, and a focal area of hyperplasia in the stomach of monkey #32M (i/m injection). Diffuse adhesions of the pleural lining to the lungs were noted in 3 of the 4 monkeys on study; these findings are not abnormal for wild-caught animals and were considered incidental to IFN-b treatment. Animal #6m also had adhesions present in the liver (between the capsule of Glisson and the diaphragm) and in the pericardium of the heart, with evidence of local inflammation in the epicardium. These tissues were not examined histologically, since the findings were considered unrelated to IFN-β exposure.

In summary, a single i/v or i/m administration of 20 MIU/kg IFN-β to cynomolgus monkeys was associated with only transient elevations in rectal body temperature. This is an expected, pharmacologic effect of type I interferons, and is not considered to be a toxic finding. There were no other overt clinical or gross pathologic signs of toxicity after a single administration of the biologic. Therefore, the NOAEL for IFN-β in the cynomolgus monkey after a single, i/v or i/m injection is $\geq$ 20 MIU/kg.

Study #XXXXXXXXXX. ***13-week repeated dose toxicity study in Sprague-Dawley Crl:CD (SD) BR rats treated with the test article interferon Beta, recombinant administered by intramuscular route at the dosage of 0, 0.25, 0.5, 1 MIU/kg/day.***

Sprague-Dawley, outbred rats were used to determine the toxicity of repeat administrations of IFN-β. Animals received daily, i/m injections of IFN-β into the thigh muscle (biceps femoris), with injections alternated daily between the right and left sides. Clinical observations were performed twice daily for mortality, and every day for signs of overt toxicity. Body weights were obtained prior to dosing at the beginning of the treatment period, then at weekly intervals for the duration of the study. Adjustments to the amount of IFN-β administered were based on changes in weekly body weights, so that the dosing remained constant throughout the study. Food consumption was monitored weekly; water consumption was not measured.

Rectal body temperatures were determined from 10 animals/sex/group using an electronic thermometer at baseline, and at 2 and 24 h following the first dose of IFN-β. Samples of urine for urinalysis were collected at study termination for 16 h in metabolic cages, after a water load of 10 ml/kg by gavage. Peripheral blood samples for hematologic and serum biochemistry profiles were obtained before terminal sacrifice from the sublingual vein, under light ether anesthesia. Serum obtained from these samples was also assayed for antibodies to both human serum albumin (HAS) present in the placebo and IFN-β formulations, and for antibody to IFN-β by RIA.

Ophthalmologic examinations were performed on both eyes of each animal at baseline, and at study termination at either week 4 or week 13. At terminal sacrifice, all animals were subjected to full necropsy, with gross pathologic evaluation of target tissues. Organs were removed, weighed, and samples preserved in 10% buffered formalin for animals in the control and high-dose groups. Any lesions present in the mid- and low-dose groups were also fixed and processed for histopathologic examination. Prior to embedding in paraffin, tissue samples were post-fixed for 30 min in XXXXXXXXXX fluid, embedded and sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Samples of femoral bone marrow (smears) were also obtained at necropsy, fixed, and stained with XXXXXXXXXX for evaluation of cellularity.

One animal (#9680F, control group) died accidentally of an ether overdose on d 30, while attempting to obtain a peripheral blood sample prior to interim sacrifice. This animal was immediately necropsied. There were no gross pathologic lesions observed and no treatment-related signs of toxicity. Histologic evaluation revealed slight inflammation in the skin and underlying injected muscle; however, this effect was noted in animals in all dose groups, including the control at terminal sacrifice and may be attributed to the trauma caused by repeated needle injections (please see below).

There were no other treatment-related signs of clinical toxicity, although several incidental changes, including transient chromodacryorrhea in one female animal each in the low and mid-dose groups, and alopecia on the cheek of one male rat in the low dose

group at approximately d 88 and continuing through study termination. This animal also developed crusting patches on both cheeks, beginning from approximately d 92 onward.

There were no apparent effects of IFN-β treatment at any dose on food consumption, body weights, or body weight gains, as compared to animals treated with the placebo control article. There were no ophthalmologic changes observed either from baseline, or between control and IFN-β-treated animals after 4 weeks of treatment. At 13 weeks, three male rats in the mid-dose group (0.5 mg/kg/d IFN-β) had developed unilateral corneal opacities. One male rat treated with 1 MIU/kg/d IFN-β had a diffuse hemorrhage of the vitreous body in the right eye on fundoscopic examination. Since these findings were unilateral in nature, occurred without relationship to the dose of the test article, and were present only in the male animals, they were considered to be incidental to the treatment with IFN-β. They are most likely a result of trauma incurred either through fighting or by daily handling procedures.

No alterations in rectal body temperature, either from baseline or as compared to control rats were observed either 2 or 24 h after IFN-β treatment on d 1 or on d 87 in any of the male test animals. Significant ($p < 0.05$, Dunnetts test) decreases in mean rectal body temperature, as compared to the control group were found for female animals dosed with either 0.25 or 0.5 MIU/kg/d at the d 87, 2 h time point. However, these changes were considered incidental to the treatment, as they were not dose-related and were within the normal range of variability for body temperature in this species.

After either 4 or 13 weeks of treatment with IFN-β, there were no significant changes in any of the hematologic parameters as compared to control animal values, with one exception. The percentage of eosinophils in peripheral blood was elevated at week 14 in 7/10 female rats treated with 0.5 MIU/kg/d, as compared to the mean value for the control female animals. However, this finding was not considered biologically significant, as it was not observed in either the high or low-dose female rats, or in the male animals at any dose of IFN-β.

There were no major findings in the clinical chemistry profiles that could be attributed to treatment with IFN-β at either the 4 or 13-week terminal sacrifices. Although several statistically significant differences in serum biochemistry profiles were noted in IFN-β-treated animals as compared to control rats, including changes in serum globulin, creatinine, AST, and glucose levels, these findings were sporadic and not related to the dose of the test article administered. There were no significant alterations in the urinalysis profiles, which could be attributed to exposure to IFN-β.

At necropsy, there were no grossly evident lesions in any organs that were related to IFN-β treatment. Slight hemorrhagic areas at the injection site were noted in several animals in both the control and the IFN-β-treated groups at both the 4 and 13-week sacrifices. These changes were present at about equal incidence and severity between the groups, and were attributed to trauma induced by repeated needle injections. Other incidental findings included several animals with focal changes or a pale appearance to the liver, congested areas in the lungs of several rats, and decreased firmness and/or size of the

testes or ovaries in rats in both control and test article-treated groups. All absolute and relative organ weights were within normal range for this strain of rat at these age and weight ranges, with the exception of the mean absolute liver weight for the high-dose male rats. This value was apparently increased from control due to a decrease in fasted body weights in this group at the 13-week time point.

No treatment-related findings were present on histologic evaluation of tissues from the female control rat (#9680) that died on study, with the exception of slight, subacute inflammatory infiltrates of mononuclear cells at the injection site, associated with local hemorrhage and muscle degeneration. These changes were present at approximately equal incidence and severity in animals of both sexes in the control and in all three IFN-β-treated groups at both the 4 and 13 week time points. A slight increase in inflammatory cells was noted in rats of both sexes treated with 1 MIU/kg/d at the interim sacrifice as compared to either the other dose groups, or to the 13-week tissue samples. All other findings were considered incidental to the IFN-β exposure.

Analysis of serum levels of anti-HSA and anti-IFN-β antibodies demonstrated that by week 5, significant titers of antibody to both proteins had developed in all groups of animals. The anti-HSA titers continued to increase over the duration of treatment, and were approximately 1.5 to 2-fold greater at 13 weeks than at the 5-week time point in both male and female animals. By contrast, anti-IFN-β antibody titers were elevated approximately 2-fold in all treated groups as compared to the values obtained for the vehicle control animals at the 5 week time point, but did not increase significantly between 5 and 13 weeks on study. Of significance, no samples were obtained from the rats prior to study initiation, to determine the background levels of anti-IFN-β activity. Blank samples, using serum from untreated animals housed in the same facility as the test animals were used to determine background levels of non-specific binding. The data are presented in the table, below:

Table XI - Serum Antibody Titers in Rats after Repeat I/M Administration of IFN-b

A. Male Rats

Dose of IFN-b (MIU/kg/d)	**Mean Serum Antibody Binding Activity (cpm) ± S.E.M.**			
	Anti-HSA		**Anti-IFN-b**	
	4 weeks	**13 weeks**	**4 weeks**	**13 weeks**
0	10593 ± 1446	16540 ± 376	5595 ± 647	4536 ± 185
0.25	15661 ± 282	16852 ± 302	10607 ± 299	10754 ± 389
0.5	12156 ± 1263	15433 ± 615	11061 ± 504	10673 ± 727
1.0	12077 ± 1042	14510 ± 503	9680 ± 592	11051 ± 444
Serum blank	809 ± 20	1039 ± 96	5368 ± 582	-not done-

B. Female Rats

Dose of IFN-b (MIU/kg/d)	Mean Serum Antibody Binding Activity (cpm) ± S.E.M.			
	Anti-HSA		Anti-IFN-b	
	4 weeks	13 weeks	4 weeks	13 weeks
0	8373 ± 1995	16592 ± 528	5634 ± 157	4732 ± 298
0.25	10825 ± 1059	17232 ± 294	10363 ± 418	10827 ± 328
0.5	11340 ± 819	16723 ± 393	10266 ± 628	10803 ± 512
1.0	8734 ± 1392	16340 ± 564	9441 ± 381	10804 ± 416
serum blank	804 ± 43	1161 ± 236	4304 ± 366	-not done-

In summary, treatment of rats with IFN-β for 13 weeks by daily, i/m injection was not associated with any obvious local or systemic toxicities. Antibody activity directed against both the test article IFN-β, and the carrier protein HSA developed after 4 weeks of treatment. However, the amount of anti-IFN-β activity in rat serum did not increase with further treatment, while the anti-HSA antibody was approximately doubled in all groups, including the control animals at study termination. The NOAEL for IFN-β in the rat by i/m administration for 13 weeks is 1.0 mg/kg/d.

Study #XXXXXXXXXX. ***13-week repeated dose toxicity study in Sprague-Dawley Crl:CD (SD) BR rats treated with the test article Interferon Beta, Recombinant administered by intravenous route at the dosages of 0, 0.25. 0.5, 1.0 MIU/kg/day.***

The toxicity of repeat, i/v administration of IFN-β was evaluated in Sprague-Dawley, outbred rats after 4 or 13 weeks of treatment. Daily, i/v injections of IFN-β were administered into the tail vein, at a rate of approximately 0.1 ml/sec and a final volume of 2.0 ml/kg. The test article was prepared by serial dilutions of the vialed IFN-β in vehicle (0.9% saline).

Comment: Dilution of the test article in saline, rather than in placebo solution (0.9% saline plus HSA) effectively dilutes the concentration of HSA in the mid and low-dose groups to 50 and 25%, respectively, of the amount in either the control or 1.0 mg/kg/d dose groups. This may partially explain the variability observed in the generation of anti-HSA antibodies after 4 and 13 weeks of treatment (please see Table , below).

Body weights were obtained prior to dosing at the beginning of the treatment period, then at weekly intervals for the duration of the study. Adjustments to the amount of IFN-β administered were based on changes in weekly body weights, so that the dosing remained constant throughout the study. Clinical observations were performed twice daily for

mortality, and every day for signs of overt toxicity. Food consumption was monitored weekly; water consumption was not measured.

Rectal body temperatures were determined from 10 animals/sex/group using an electronic thermometer at baseline, and at 2 and 24 h following the first dose of IFN-β, and again after 13 weeks of treatment (d 89 for males, d 85 for females). Samples of urine for urinalysis were collected at study termination for 16 h in metabolic cages, after a water load of 10 ml/kg by gavage. Peripheral blood samples for hematologic and serum biochemistry profiles were obtained before terminal sacrifice from the sublingual vein, under light ether anesthesia. Serum obtained from these samples was also assayed for antibodies to both human serum albumin (HSA) present in the placebo and IFN-β formulations, and for antibody to IFN-β by RIA.

Additional blood and urine samples were obtained and analyses performed at week 9 (days 57 and 60) on all surviving animals at these time points. Ophthalmologic examinations were performed on both eyes of each animal at baseline, and at study termination at either week 4 or week 13.

At terminal sacrifice, all animals were fasted overnight, euthanized by i/p overdose with sodium pentobarbital solution, and subjected to full necropsy with gross pathologic evaluation of target tissues. Organs were removed, weighed, and samples preserved in 10% buffered formalin for animals in the control and high-dose groups. Any lesions present in the mid- and low-dose groups were also fixed and processed for histopathologic examination. Prior to embedding in paraffin, tissue samples were post-fixed for 30 min in XXXXXXXXXX fluid, embedded and sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Samples of femoral bone marrow (smears) were also obtained at necropsy, fixed, and stained with XXXXXXXXXX for evaluation of cellularity. Any animal dying, or sacrificed moribund prior to completion of IFN-β treatment was also evaluated by both gross and histopathologic examination following a full necropsy.

There were no mortalities in any of the animals treated during the first four weeks on study. Numerous animals from all groups, including the control group died during the later stage of the treatment phase (weeks 5-13). Three control male rats died during week 10 of treatment, and one control female rat died at d 71 (week 11). Two animals of each sex in the group treated with 0.25 MIU/kg IFN-β/day died during week 7, and one male rat in this group died on d 41 (week 6). One additional male died at d 66 and two females in this group died on d 60.

In the group receiving 0.5 mg/kg/d IFN-β, 5/10 male rats died during week 6 and one male died on d 44 (week 7). Two female rats in this group died on days 34 and 37. Mortality in the highest dose group (1.0 MIU/kg/d) was first observed in two female rats during week 6, with 5/10 male rats dying during week 8, and one male rat dying at weeks 7, 9, and 10. At study termination, only 2/10 male rats and 6/10 female rats initially treated remained alive in this group.

Two female rats (#9908 and #9909) in the 0.25 MIU IFN-β/kg/d dose group died accidentally of an ether overdose on d 60, while attempting to obtain a peripheral blood sample. These animals were immediately necropsied. There were no gross pathologic lesions observed and no treatment-related signs of toxicity. There were no overt clinical signs of toxicity present in these two animals prior to the accidental death. No clinical signs and no overt lesions were present in animal #9912F in this group as well; however, several organs were missing, having been cannibalized so no definitive cause of death could be assigned.

All other early mortalities had clinical evidence of respiratory distress (*i.e.* rales, dyspnea, and polypnea). Histologic evaluation revealed purulent pneumonia in the lungs of all of these animals, and in some cases visible colonies of bacteria were present on microscopic examination. Several animals also had acute and/or lymphocytic inflammatory changes in other organs, including the heart, kidneys, thymus, aorta, liver, or spleen. The cause of the early deaths on study was therefore attributed to pneumonia, secondary to bacterial infection from an external source (please see below).

All of the animals either sacrificed moribund or found dead had ulcerative lesions on the tail, in the area of the injection site(s). Gross evaluation revealed the presence of pus, which was confirmed histologically as purulent inflammation extending into the dermis, and sporadically associated with colonies of bacteria. Swelling around the injection site(s), as well as necrosis of the tail were also present, especially in the mid- and high-dose groups. All other changes observed in these early mortalities were either spontaneous, incidental pathologies or the result of post-mortem autolysis, and were not related to treatment with the test article.

One rat in the group treated with 0.5 MIU/kg/d IFN-β (#9951F, died d 37) had a cyst present in the colon at necropsy, which was revealed on microscopic evaluation to be a mucosal adenoma. This finding was considered to be incidental to the treatment with IFN-β.

Clinical signs of toxicity included the presence of ruffled coat(s), red/brown nasal discharge, and thinness in all of the surviving animals in the IFN-β-treated groups. Respiratory difficulties were present in several animals in all groups, including the control, and consisted of dyspnea with or without rales, and polypnea. Three male rats in the mid-dose group and one rat of each sex in the group receiving 1.0 MIU/kg/d IFN-β displayed ataxic gait, suggestive of imbalances in maintaining equilibrium. Other incidental clinical findings included alopecia on the top of the head of one female rat in the control and the 0.5 MIU IFN-β/kg/d dose groups, chromodacryorrhea in one female in the highest dose group, and apparent cyanosis in one female rat in the mid-dose group and two females in the group treated with 1.0 MIU/kg/d IFN-β. None of these findings were considered by the contract laboratory, the examining pathologist, or the sponsor to be related to the treatment with the test article.

Overall, there were no significant, treatment-related changes in either body weight or total body weight gain in the IFN-β-treated rats, as compared to the control animals with

one exception. At week 6 on study, the mean body weight for male rats in the group treated with 0.5 MIU/kg/d IFN-β was statistically significantly decreased from the control animals. However, this group of animals also had a number of rats die during week 6 of treatment, which may account for the decrease in mean body weight at this time point. Mean food consumption did not significantly differ between groups throughout the duration of the study, although lower values were reported for food consumption when deaths occurred. Rectal body temperatures were not affected by treatment with IFN-β, at either the 2 or 24 h time points measured after the first or final dose.

At the 4 week interim sacrifice, there were no significant differences from control values in the hematologic profiles for the groups of animals treated with 0.25, 0.5, or 1.0 MIU/kg/d IFN-β. At week 9, the surviving rats in the highest dose group (1.0 MIU/kg/d) had elevations in platelet numbers, total leukocyte counts, and the percentage of neutrophils, with a corresponding decrease in the percentage of lymphocytes as compared to the control rats. However, these differences were not statistically significant in the male animals, due to the small number of survivors (2). These findings were significantly different from the control values in the female animals at this time point. The female rats in both the mid- and high-dose groups also displayed significant decreases in erythrocyte counts, hemoglobin, and hematocrit values as compared to the control group.

An additional finding at the 9 week time point was an increase in the mean value for prothrombin time in male rats treated with 0.5 MIU/kg/d, as compared to the control group. This finding occurred only in the mid-dose group, and was not considered related to treatment with the test article. No similar change was observed in the female rats in this dose group.

By week 13, there were several statistically significant changes in hematologic parameters for the female rats in all of the IFN-β-treated groups, as compared to the control animals. Most notably, these findings included significant decreases in total leukocyte counts and percentage of neutrophils, with a concomitant increase in the percentage of lymphocytes. However, on closer evaluation of the data, the mean values for these parameters in female rats in the control group are elevated outside of the normal range for this strain. The statistically significant findings in the IFN-β-treated groups, therefore, are most likely due to this elevation, and are not of toxicologic significance.

Clinical chemistry profiles demonstrated significant increases from control values in albumin levels, with a concomitant decrease in the serum globulin profile and an increased A:G ratio in male rats treated with either 0.5 or 1.0 MIU/kg/d IFN-β at the week 4 interim sacrifice, and in female rats at terminal sacrifice following treatment with either 0.25 or 1.0 MIU/kg/d IFN-β. Fractionation of the globulin proteins revealed significant decrease as compared to control in the mean value for the α1-globulin fraction obtained for the female rats in the low dose group, and animals of both sexes in the high dose group at the week 4 sacrifice only. A significant increase in the γ-globulin fraction as compared to the control value was detected at 9 weeks in the male rats treated with 1.0

MIU/kg/d of the test article, while a decrease was observed in females in this same group at week 13. This finding was considered incidental to treatment with the test article, since the γ-globulin fraction was abnormally elevated in two female rats (#9865 and #9871) in the control group. Serum creatinine levels were elevated in the high-dose male rats at week 9 and in the female mid-dose group at week 13 when compared to control values, however, this finding was due to an abnormally high level in male rat #9967 (3.43 mg/dL). The other male rats in this group all had creatinine values within the normal limits for this strain of rat. At terminal sacrifice, the creatinine value for this rat had resolved to within normal limits, and the mean value for the surviving male rats in the group treated with 0.5 MIU/kg/d IFN-β had creatinine values which were significantly less than control values, but still within normal limits. Other changes observed in the female rats in this group included significant increases in serum electrolyte (sodium, potassium, calcium, and chloride) levels at the week 13 time point, when compared to control values. However, these changes were still within normal limits for this strain of rats, and do not constitute an abnormal or toxicologic finding. Similar changes were not observed in this group at any other time point, nor in the male rats treated with IFN-β at any time on study.

No ophthalmologic findings were included in the final study report. No significant differences in urinalysis parameters between control and IFN-β-treated rats were found at the three time points evaluated on study (week 4, week 9, and at terminal sacrifice at week 13).

At necropsy, there were no overt, treatment-related signs of toxicity, either in the animals at the 4 week interim sacrifice, or in the surviving rats at study termination at 13 weeks. Incidental findings in the rats after 4 weeks of IFN-β treatment included unilateral hydronephrosis and decreased size of the right kidney, and a slight decrease in pituitary size in one control, male rat (#9859M), and in one male in the high-dose group (rat #9977M). Increased prostate size with evidence of slight hemorrhage was observed in two male rats treated with 0.25 MIU/kg/d IFN-β (animals #9900M and #9902M), and in one rat in the 1.0 MIU/kg/d dose group (animal #9982M). One male rat in the mid-dose group (animal #9943M) had a focal, whitish area present in the liver on gross evaluation, and one female rat (animal #5F) in the high-dose group had a focal area in the median lobe described as yellowish and firm. Pale livers were noted in one male and one female rat each, in the highest dose group (animals #9983M and #9999F, respectively), and in one female rat in the low and mid-dose groups (animals #9921F and #9962F, respectively). Female rat #9922F treated with 0.25 MIU IFN-β/kg/d had a dark, moderately elevated area with a mucoid exudate present on the back, in the subcutaneous tissue. There were no other macroscopically visible lesions in any of the test or control animals at the 4 week time point.

After 13 weeks of treatment, macroscopic findings at necropsy included several animals in all groups, including the control, with pleural adhesions, abscesses, or purulent areas in the lungs, of slight to moderate severity. These findings were observed in 2/7 male and 3/9 female rats in the control group, 1/6 male rats in the group treated with 0.25 MIU IFN-β/kg/d, 2/6 male rats in the mid-dose group, and ¼ male and 2/6 female rats

receiving 1.0 MIU/kg/d IFN-β. Histologically, these changes represented thickening of the pleura, which was slight to moderate in severity and was accompanied in many cases by perivascular, lymphoid infiltrates and cuffing. Focal areas of alveolar histiocytosis, representing proliferation of macrophages and type II pneumocytes, and subacute inflammatory changes were also present in animals in all groups, including the controls. Microscopic evaluation of the injection site revealed evidence of slight to severe tissue trauma, including perivascular hemorrhage and inflammation, moderate to severe suppurative changes around the needle tract, and necrosis of the dermis and epidermis. These findings were present in animals in all groups, including the control, with no relation of either incidence or severity to the dose of the test article. All other findings, both macroscopic and histologic, were considered incidental to treatment with IFN-β.

Analysis of serum levels of anti-HSA and anti-IFN-β antibodies demonstrated that after 4 weeks of treatment, increased titers of antibody to both proteins had developed in all groups of animals. The anti-HSA titers continued to increase over the duration of treatment, and were approximately 1.5 to 2-fold greater at 9 and 13 weeks, than at the 4-week time point in both male and female animals. Interestingly, the values obtained for the anti-HSA titers in the rats treated with 0.25 and 0.5 MIU/kg/d IFN-β were lower than the antibody titers generated in the animals treated with the placebo control or with the highest dose of IFN-β. This finding results from the procedure used to dilute the samples prior to administration; serial dilutions of IFN-β were done using 0.9% sterile saline, rather than the placebo control solution as the diluent. This practice effectively diluted the concentration of HSA in the mid-and low-dose groups by 50% and 25%, respectively, from what was present in the control and 1.0 MIU/kg/d IFN-β doses.

Antibody titers to IFN-β were only slightly elevated, as compared to the values obtained for the vehicle control animals at the 5 week time point, and did not increase significantly any further at the 9 and 13 week time points. Blank samples, using serum from untreated animals housed in the same facility as the test animals were used to determine background levels of non-specific binding. The data are presented in the table, below:

Table XII -Serum Antibody Titers in Rats after Repeat I/V Administration of IFN-b

A. Male Rats

Dose of IFN-b (MIU/kg/d	Mean Serum Antibody Binding Activity (cpm) ± S.E.M.					
	Anti-HSA			Anti-IFN-b		
	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks
0	620 ± 54	4828 ± 2076	5658 ± 2922[a]	4587 ± 109	4531 ± 148	4679 ± 125
0.25	596 ± 54	655 ± 69[a]	715 ± 77[b]	5203 ± 192	5437 ± 202	5025 ± 281
0.5	636 ± 36	855 ± 181[d]	1067 ± 276[d]	4931 ± 103	5077 ± 296	5561 ± 493
1.0	692 ± 39	2811 ± 956[c]	14298±3759[d]	5242 ± 305	5654 ± 281	5391 ± 531
Serum blank	592 ± 119			4413 ±124		

[a] n = 7
[b] n = 6
[c] n = 5
[d] n = 4

A. Female Rats

Dose of IFN-b (MIU/kg/d	Mean Serum Antibody Binding Activity (cpm) ± S.E.M.					
	Anti-HSA			Anti-IFN-b		
	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks
0	843 ± 44	1063 ± 150[b]	2167 ± 784[a]	4631 ± 71	4424 ± 83	4463 ± 115
0.25	867 ± 55	877 ± 67[c]	1361 ± 499[c]	5066 ± 335	5132 ± 322	5389 ± 388
0.5	763 ± 36	2959 ± 1955[b]	2028 ± 1189[b]	4953 ± 218	6170 ± 210	6591 ± 314
1.0	5037 ± 1856	11420±2900[c]	9649 ± 2118[c]	5765 ± 177	6657 ± 241	6389 ± 232
Serum blank	765 ± 24			4513 ± 24		

[a] n = 9
[b] n = 8
[c] n = 6

In summary, daily, i/v injection of rats with IFN-β for 13 weeks was not associated with any obvious local or systemic toxicities related to treatment with the test article. Mortalities were present in all groups, including the placebo control, and were secondary to infection at the injection site and subsequent bacterial colonization and pneumonia in the lung. Antibody activity directed against both the test article IFN-β, and the carrier protein HSA developed after 4 weeks of treatment. However, the amount of anti-IFN-β activity in rat serum did not increase with further treatment, while the anti-HSA antibody was approximately doubled in all groups, including the control animals at study termination. Because of the increased mortalities observed in all groups, no NOAEL can

be determined for IFN-β in the rat, following i/v administration for 13 weeks. The NOAEL for IFN-β in the rat after 4 weeks of i/v injections is 1.0 MIU/kg/d.

Study #XXXXXXXXXX. ***13-Week repeated dose toxicity study in cynomolgus monkeys treated with the test article Interferon Beta, Recombinant administered by intramuscular route at the dosages of 0, 0.25. 0.5, and 1 MIU//kg/day.***

The purpose of this study was to evaluate the toxicity of IFN-β in a pharmacologically responsive species, the cynomolgus monkey. Animals received daily, i/m injections of IFN-β into the thigh muscle (biceps femoris), with injections alternated daily between the right and left sides. Adjustments to the amount of IFN-β administered were based on changes in weekly body weights, so that the dosing remained constant throughout the study. The test article was prepared by serial dilutions of the vialed IFN-β in vehicle (0.9% saline).

Comment: Dilution of the test article in saline, rather than in placebo solution (0.9% saline plus HSA) effectively dilutes the concentration of HSA in the mid- and low-dose groups to 50 and 25%, respectively, of the amount in either the control or 1.0 mg/kg/d dose groups. This may partially explain the variability observed in the generation of anti-HSA antibodies after 4 and 13 weeks of treatment (please see Table XIII, below).

Body weights were obtained at baseline on day –7 and on day 0, prior to dosing, then at weekly intervals for the duration of the study. Clinical observations were performed twice daily for mortality, and every day for signs of overt toxicity. Food and water consumption were not measured; however, any abnormal food consumption or evidence of inappetence was recorded. Animals were allowed free access to a ration of food equivalent to 3-4% of their body weights daily, and supplemented with fresh fruit twice weekly. Filtered drinking water was available *ad libitum*, through an automatic watering system.

Rectal body temperatures were determined using an electronic thermometer at baseline, immediately prior to dosing on d 0, and at 2 and 24 h following the first dose of IFN-β. Body temperatures were measured on d 27/28 at these same time points (24 h was just before the 4-week interim sacrifice), and again in the remaining animals after 13 weeks of treatment. Electrocardiograms were recorded on each animal (under Ketamine and diazepam anesthesia) at baseline (week –1), and after 4 and 13 weeks of treatment with IFN-β. Ophthalmologic examinations were performed on both eyes of each animal at baseline, and at study termination at either week 4 or week 13.

Samples of urine for urinalysis and fecal samples for occult blood were collected from fasted monkeys for 16 h in metabolic cages at baseline, d 22/23 (week 4 on study), and prior to study termination (d 86/87), after a water load of 20 ml/kg. Peripheral blood samples for hematologic and serum biochemistry profiles were obtained at these same time points from the cephalic vein, after the animals had been fasted overnight. Serum obtained from these samples was also assayed for antibodies to both human serum

albumin (HSA) present in the placebo and IFN-β formulations, and for antibody to IFN-β by RIA.

At terminal sacrifice, monkeys were anesthetized with sodium pentobarbital, weighed, and exsanguinated through the femoral arteries. All animals were subjected to full necropsy, with gross pathologic evaluation of target tissues. Organs were removed, weighed, and individual organ weight:fasted body weight ratios were calculated. Tissue samples were obtained from animals in all treatment groups, and preserved in 10% buffered formalin, fixed, and processed for histopathologic examination. Prior to embedding in paraffin, tissue samples were post-fixed for 30 min in XXXXXXXXXX fluid, embedded and sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Samples of sternal bone marrow (smears) were also obtained at necropsy, fixed, and stained with XXXXXXXXXX for evaluation of cellularity.

There were no early mortalities on study; all animals survived until scheduled sacrifice. There were no overt signs of clinical toxicity present in any of the control or IFN-β-treated animals at any time point on study that could be related to treatment with the test article. Body weights and body weight gain over the duration of the study were not significantly different in the groups of animals treated with IFN-β, as compared to the controls. Reduced food intake, soft stools, and/or diarrhea were observed transiently at various times on study in monkeys in all groups, including the controls. One animal (#1672M) in the group treated with 0.25 MIU/kg/d IFN-β had more frequent episodes of loose or watery stool than the other monkeys. These findings were not associated with a decrease in body weight over the duration of the treatment period, and were considered incidental to treatment with IFN-β.

Alopecia, most notably located on the anterior and/or posterior limbs was noted in one monkey in each of the dosing groups, including animal #1649F in the control group. There was no relationship of this finding to either the dose or duration of IFN-β treatment; alopecia is occasionally observed in untreated monkeys as well, so these findings were not considered related to treatment with the test article.

No ophthalmologic abnormalities were detected in any of the animals on study, either at the baseline evaluation or after 4 or 13 weeks of treatment with the placebo or IFN-β. Evaluation of the EKG profiles did not show any changes in either the heart rate or electrocardiographic patterns that were related to treatment with the test article. Minor disturbances in ventricular conduction (*i.e.* incomplete right bundle block, alteration of intraventricular conduction) were observed sporadically in several animals in both the control and IFN-β-treated groups. These alterations are not uncommon in wild-caught monkeys, and were assumed to result from spontaneous pathology in the heart. The findings were considered incidental to treatment with the test article.

Mean values for rectal body temperatures were elevated by 0.5 to 0.7^{o}C in the groups of monkeys treated with IFN-β, when compared to the control group at 2 h post-dosing on day 0. The increases were statistically significant for the animals treated on d 0 with either 0.5 or 1.0 MIU/kg IFN-β, when compared to the control monkeys ($p \leq 0.05$,

ANOVA). However, the mean value for the control animals at the 2 h time point was decreased by approximately 0.3°C from the values obtained for this group at baseline. When the rectal body temperatures for the animals in the IFN-β-treated groups at 2 h post-dosing were compared to the baseline values, there were no significant differences found. The findings at 2 h between the control and the mid- and high-dose groups actually reflect a decrease in rectal body temperatures in the control animals from the values obtained at baseline, and are not considered biologically meaningful. At 24 h after the initial treatment with IFN-β, rectal body temperatures in animals in all groups had decreased to baseline levels, and were not significantly different from the values obtained for the control monkeys at the week 4 and week 13 measurements.

Hematologic profiles were not appreciably altered from baseline, or when compared to the values obtained for the control monkeys in any of the groups of animals treated with IFN-β by i/v injection for 4 or 13 weeks. One minor finding at the week 4 time point however, was an approximate 9 – 10% increase in the percentage of lymphocytes, and a concomitant 8 – 11% decrease in the percentage of neutrophils with no change in total leukocyte counts in the animals in all groups, regardless of treatment with IFN-β. However, these values were not outside the normal ranges for wild-caught monkeys, and were primarily due to an apparent, transient decrease in differential cell percentages in the control group. At the 4-week time point, erythrocyte sedimentation rates (ESRs) were elevated 3 to 5-fold at 2 h after dosing than at the 1 h measurement, with an apparent relationship to the dose of IFN-β administered. However, this finding was also observed in all groups at the baseline measurement, and was not present after 13 weeks on study. The biologic significance of the elevated ESRs is unknown at the present time. Platelet counts and coagulation profiles (prothrombin time) were not affected by IFN-β treatment at any time point on study.

Comment: Other interferon-β preparations have demonstrated significant decreases in platelet and erythrocyte counts, elevations in ESRs and total leukocyte counts, and prolongation of coagulation times after repeated s/c or i/m treatment of Rhesus monkeys with doses as high as 10 MIU/kg/d. The NOAEL for the hematologic effects with these other preparations has been approximately 1.0 MIU/kg/d, which was the highest dose tested in the present study.

There were no definitive, treatment-related changes in clinical chemistry profiles for animals treated with 0.25, 0.5, or 1.0 MIU IFN-β/kg/d for either 4 or 13 weeks, as compared to either the placebo control group, or to baseline values. Slight elevations in the alkaline phosphatase, albumin levels and A:G ratios, with concomitant decreases in the group mean globulin values as compared to baseline were observed at 4 and 13 weeks in all groups, including the control animals. These effects appeared to be related to both the dose and duration of IFN-β treatment, however, they were not outside of normal limit values for this strain of monkeys. Serum ALT levels were elevated, although not statistically significantly, by approximately 2-fold as compared to either the control group or the baseline values for monkeys treated for 13 weeks with 0.25 MIU/kg/d IFN-β. This finding may have resulted from animal #1637F, which had a final value of 286 U/dL. However, the mean values were still within normal limits for macaque monkeys, and the

slight elevations in serum ALT, both in this individual animal and for the entire group, were not considered related to the treatment with IFN-β. Other incidental changes included transient increases in serum glucose and decreases in β-globulin levels in animals in the low- and mid-dose groups at week 4, a slight increase in blood urea nitrogen at week 4 in the mid-dose group, and a decrease in inorganic phosphorus levels as compared to control in the monkeys treated with 0.25 MIU/kg/d IFN-β for 4 weeks. All of these changes had resolved to either baseline or no significant differences from the control group at the time of study termination.

Urinalysis profiles were not significantly different between the control and IFN-β-treated monkeys. Several female animals in all of the dose groups had 3^+ to 4^+ scores for blood in the urine at either baseline, the 4-week, and/or the 13-week time point. These findings were correlated with onset of menses in these animals (see above), and were considered incidental to the treatment.

At necropsy, no treatment-related findings were evident on gross pathological examination of the IFN-β-treated monkeys. Areas of hemorrhage in the subcutaneous tissue at the injection site(s) were present in animals in both the control and the IFN-β-treated groups, at both the 4- and 13-week terminal sacrifices. These findings were most likely related to tissue trauma induced by multiple, i/m injections and were not related to the pharmacologic activity of the test article. Other observations included slight increases in the size of the spleen, multifocal, pale nodules in the liver, and parasitic nodules in the colon, cecum, and large intestine of several monkeys in all groups, including the control, at the 4- and 13-week sacrifices. There were no significant differences in either absolute, or relative organ weights for the monkeys treated with IFN-β, as compared to the control animals. Incidental findings included juvenile appearance of the testes, prostate, and epidiymides in many of the male monkeys, and increased size and/or cysts present in the ovaries in a few female animals at terminal sacrifices, indicating that these monkeys had not yet reached sexual maturity. These observations were confirmed upon histologic evaluation of the tissues, which demonstrated aspermatogenesis and aspermia in the male sex organs, and an immature, juvenile appearance to the prostate samples from the male monkeys, and corpora luteae in the ovaries of several female animals.

On histologic evaluation, there were no pathologic findings that could be directly related to treatment with IFN-β, aside from findings at the injection site(s). These included interstitial hemorrhage, inflammatory infiltrates, and lymphoid aggregates, with evidence of muscle degeneration, fibrosis, and repair(s). Injection site changes were present in animals in all groups, including the controls at approximately equal incidence and severity, both at the 4- and the 13-week sacrifices. Occasional inflammatory changes were also present in the epineural area of the sciatic nerve bundles present in the injected region. These findings are considered secondary to trauma induced by repeated i/m injections, and are not related to the pharmacologic activity of IFN-β.

Sub-acute to chronic, inflammatory changes, including predominantly lymphoid infiltrates and aggregates were present in the stomach, pancreas, salivary glands, small,

and large intestines, kidney, and urinary bladder of several monkeys in all groups, including the placebo control. Other findings included interstitial nephritis, glomerulosclerosis, and/or inflammation of the renal pelvis, of slight to mild severity in animals in all 4 groups, including the control. These findings were not related to treatment with the test article, since they were present in approximately equal incidence and severity in all groups, and at both time points on study.

Microscopic findings in the liver included multifocal areas of hepatocellular degeneration and/or necrosis accompanied by mononuclear cell infiltrates, and slight to moderate inflammatory changes in the periportal regions. Vacuolization of hepatocytes, accompanied by evidence of fatty deposition in the liver were also present in several monkeys in all groups, including the controls. Histopathologic changes were also present in the lungs of animals in all groups, and included perivascular and peribronchiolar lymphoid cuffing, inflammation and/or thickening of the pleural wall, and accumulation of pigment-laden macrophages. Similar, sub-acute inflammatory changes were also present in the tracheas of animals in all four treatment groups. These findings were slight to mild in severity, occurred at approximately equal incidence between the groups and at the 4- and 13-week sacrifices, and were not considered related to IFN-β treatment.

Other, incidental findings included slight to moderate degenerative and/or inflammatory changes in the heart, consisting of fibrosis in either the pericardium or epicardium, pigmentation in the myocardium, and degenerative changes in the myocardium, with or without inflammation present. These findings were present in 3/3 control and mid-dose females, 2/3 females in the low dose group, and one animal of each sex in the high-dose group at the 4-week sacrifice. After 13 weeks of IFN-β treatment, changes in the heart were present in 2/3 male and 2/3 female, control monkeys, 3/3 males and 1/3 females in the 0.25 MIU/kg/d dose group, and in 2/3 male and 3/3 female monkeys each, in the mid- and high-dose groups. These findings are not uncommon in wild-caught monkeys, and were considered incidental to treatment with IFN-β.

Analysis of serum levels of anti-HSA and anti-IFN-β antibodies demonstrated that after 4 weeks of treatment, 1.3 to 5.5-fold increases in anti-HSA binding activity had developed in all groups of either control or IFN-β-treated animals, as compared to the baseline values for these monkeys. Anti-HSA titers were not appreciably increased further in the mid- and high-dose male monkeys, and in all groups of female monkeys at the 13-week time point, as compared to the values obtained at the 4-week sacrifice. By contrast, the anti-HSA titers were dramatically increased at 13 weeks in 2/3 male monkeys receiving 0.25 MIU/kg/d IFN-β, and by approximately 2-fold in the control male monkeys at this time point, as compared to either the baseline or 4-week values.

Antibody titers to IFN-β were elevated by 1.4 to 2-fold over baseline after 4 weeks of treatment, in all groups of monkeys receiving treatment with the test article. Anti-IFN-β titers did not increase significantly any further in animals of either sex at the 13-week time points. These data are presented in the table, below:

Table XIII - Serum Antibody Titers in Cynomolgus Monkeys after Repeat I/V Administration of IFN-b

A. Male Monkeys

Dose of IFN-b (MIU/kg/d	Mean Serum Antibody Binding Activity (cpm) ± S.E.M.					
	Anti-HSA			Anti-IFN-b		
	Baseline	4 weeks	13 weeks	Baseline	4 weeks	13 weeks
0	625 ± 31	799 ± 121	1268 ± 284[a]	5936 ± 261	6158 ± 283	6726 ± 564
0.25	638 ± 31	2647 ± 940	12116±5355[a,b]	6265 ± 382	11704 ± 119	10921 ± 1777
0.5	676 ± 37	2419 ± 659	1416 ± 434[a]	7326 ± 326	12158 ± 79	12342 ± 310
1.0	884 ± 192	4939 ± 2779	1071 ± 39[a]	6245 ± 341	12398 ± 219	11900 ± 256
Serum blank	Not done					

[a] n = 3

[b] 2/3 animals had high (>16,000) anti-HSA antibody titers, 3rd animal titer was 1441

B. Female Monkeys

Dose of IFN-b (MIU/kg/d	Mean Serum Antibody Binding Activity (cpm) ± S.E.M.					
	Anti-HSA			Anti-IFN-b		
	Baseline	4 weeks	13 weeks	Baseline	4 weeks	13 weeks
0	538 ± 16	794 ± 59	860 ± 164[a]	6881 ± 276	6410 ± 220	7001 ± 210
0.25	561 ± 20	1623 ± 385	1399 ± 467[a]	7029 ± 294	9973 ± 92	10118 ± 242
0.5	567 ± 24	1741 ± 365	2317 ± 925[a]	7320 ± 333	10399 ± 73	10462 ± 108
1.0	626 ± 17	1658 ± 231	1126 ± 226[a]	7398 ± 257	10382 ± 142	10396 ± 170
Serum blank	Not done					

[a] n = 3

In summary, daily i/m injection of cynomolgus monkeys with IFN-β were associated with only transient elevations in rectal body temperature, and inflammatory reactivity at the injection site. The elevation in body temperature is an expected, pharmacologic, effect of type I interferons, and is not considered to be a toxic finding. Injection site reactions were present in approximately equal incidence and severity in all groups, including the placebo controls. There were no other overt clinical or gross pathologic signs of toxicity after up to 13 weeks of administration of the biologic. Antibodies to both interferon and the carrier protein were detected as early as 4 weeks in all groups of IFN-β-treated monkeys. However, neutralizing antibody activity was not measured, so no conclusions regarding the effects of antibody development on the toxicity can be made. The apparent NOAEL for IFN-β in the cynomolgus monkey after repeated, i/m injection for 4 or 13 weeks is ≥ 1.0 MIU/kg.

Study #XXXXXXXXXX. ***13-Week repeated dose toxicity study in cynomolgus monkeys treated with the test article Interferon Beta, Recombinant administered by intravenous route at the dosages of 0, 0.25. 0.5, and 1 MIU//kg/day.***

The purpose of this study was to evaluate the toxicity of IFN-β in a pharmacologically responsive species, the cynomolgus monkey. Daily, i/v injections of IFN-β were administered into the cephalic vein, alternating between the right and left sides daily, at a rate of approximately 0.1 ml/sec and a final volume of 0.5 ml/kg. The test article was prepared by serial dilutions of the vialed IFN-β in vehicle (0.9% saline).

Comment: Dilution of the test article in saline, rather than in placebo solution (0.9% saline plus HSA) effectively dilutes the concentration of HSA in the mid- and low-dose groups to 50 and 25%, respectively, of the amount in either the control or 1.0 mg/kg/d dose groups. This may partially explain the variability observed in the generation of anti-HSA antibodies after 4 and 13 weeks of treatment (please see Table XIV, below).

Body weights were obtained at baseline on day –7 and on day 0, prior to dosing, then at weekly intervals for the duration of the study. Adjustments to the amount of IFN-β administered were based on changes in weekly body weights, so that the dosing remained constant throughout the study. Clinical observations were performed twice daily for mortality, and every day for signs of overt toxicity. Food and water consumption were not measured; however, any abnormal food consumption or evidence of inappetence was recorded. Animals were allowed free access to a ration of food equivalent to 3-4% of their body weights daily, and supplemented with fresh fruit twice weekly. Filtered drinking water was available *ad libitum*, through an automatic watering system.

Rectal body temperatures were determined using an electronic thermometer at baseline, immediately prior to dosing on d 0, and at 2 and 24 h following the first dose of IFN-β. Body temperatures were measured on d 27/28 just before the 4-week interim sacrifice, and again in the remaining animals after 13 weeks of treatment. Electrocardiograms were recorded on each animal (under Ketamine and diazepam anesthesia) at baseline (week –1), and after 4 and 13 weeks of treatment with IFN-β. Ophthalmologic examinations were performed on both eyes of each animal at baseline, and at study termination at either week 4 or week 13.

Samples of urine for urinalysis and fecal samples for occult blood were collected from fasted monkeys for 16 h in metabolic cages at baseline, d 22/23 (week 4 on study), and prior to study termination (d 86/87), after a water load of 20 ml/kg. Peripheral blood samples for hematologic and serum biochemistry profiles were obtained at these same time points from the cephalic vein, after the animals had been fasted overnight. Serum obtained from these samples was also assayed for antibodies to both human serum albumin (HSA) present in the placebo and IFN-β formulations, and for antibody to IFN-β by RIA.

At terminal sacrifice, monkeys were anesthetized with sodium pentobarbital, weighed, and exsanguinated through the femoral arteries. All animals were subjected to full necropsy, with gross pathologic evaluation of target tissues. Organs were removed, weighed, and individual organ weight:fasted body weight ratios were calculated. Tissue samples were obtained from animals in all treatment groups, and preserved in 10% buffered formalin, fixed, and processed for histopathologic examination. Prior to embedding in paraffin, tissue samples were post-fixed for 30 min in XXXXXXXXXX fluid, embedded and sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Samples of femoral bone marrow (smears) were also obtained at necropsy, fixed, and stained with XXXXXXXXXX for evaluation of cellularity.

There were no early mortalities on study; all animals survived until scheduled sacrifice. There were no overt signs of clinical toxicity present in any of the control or IFN-β-treated animals at any time point on study that could be related to treatment with the test article.

Body weights and body weight gain over the duration of the study were not significantly different in the groups of animals treated with IFN-β, as compared to the controls. Reduced food intake, soft stools, and/or diarrhea were observed transiently at various times on study in monkeys in all groups, including the controls. These findings were not associated with a decrease in body weight over the duration of the treatment period, and were considered incidental to treatment with IFN-β.

Alopecia, most notably located on the anterior and/or posterior limbs was noted in several monkeys, including animal #1734M in the control group. There was no relationship of this finding to either the dose or duration of IFN-β treatment; alopecia is occasionally observed in untreated monkeys as well, so these findings were not considered related to treatment with the test article.

Several female monkeys (control animals #1689F and #1731F, low-dose animals #1697F and #1703F, and mid-dose monkey #1691F) showed normal signs of menorrhea during the course of the study. No ophthalmologic abnormalities were detected in any of the animals on study, either at the baseline evaluation or after 4 or 13 weeks of treatment with the placebo or IFN-β.

Evaluation of the EKG profiles did not show any changes in either the heart rate or electrocardiographic patterns that were related to treatment with the test article. Minor fluctuations in ventricular conduction or repolarization phases, or slight alterations in the ECG complexes were observed sporadically in several animals in both the control and IFN-β-treated groups. These alterations are not uncommon in anesthetized monkeys, and were considered incidental to treatment with the test article.

Mean values for rectal body temperatures were elevated in all IFN-β-treated groups of animals by 0.5 to 0.7^{o}C from baseline, or as compared to the control monkeys at 2 h after treatment with IFN-β. Rectal body temperatures had decreased to baseline levels by 24 h

post-treatment with IFN-β, and were not significantly different from the values obtained for the control monkeys at the week 4 and week 13 measurements.

Hematologic profiles were not appreciably altered from baseline, or when compared to the values obtained for the control monkeys in any of the groups of animals treated with IFN-β by i/v injection for 4 or 13 weeks. One minor finding at the week 4 time point, however, was a slight increase in the percentage of lymphocytes, with a concomitant decrease in the percentage of neutrophils and no change in overall, total leukocyte counts in the animals treated with either 0.5 or 1 MIU/kg/d IFN-β. Although statistically significantly different from control, these values were not outside the normal ranges for wild-caught monkeys, and were primarily due to an apparent, transient decrease in differential cell percentages in the control group.

There were no treatment-related changes in clinical chemistry profiles for animals treated with 0.25, 0.5, or 1.0 MIU IFN-β/kg/d for either 4 or 13 weeks, as compared to the placebo control group. A slight elevation in the mean total cholesterol level as compared to the control value was observed for the mid-dose group at the 4-week sacrifice; this finding may have resulted from animal #1713F, who had a final value of 181 mg/dL. However, the mean values were still within normal limits for macaque monkeys, and the slight elevations in cholesterol, both in this individual animal and for the entire group, were not considered related to the treatment with IFN-β. Urinalysis profiles were not significantly different between the control and IFN-β-treated monkeys. Several female animals in the control, low- and mid-dose groups had 4+ scores for blood in the urine at either baseline and/or the week 4 time point; these findings were correlated with onset of menses in these animals (see above), and were considered incidental to the treatment.

At necropsy, no treatment-related findings were evident on gross pathological examination of the IFN-β-treated monkeys. Areas of hemorrhage in the subcutaneous tissue at the injection site(s) were present in several animals in both the control and the IFN-β-treated groups, at both the 4- and 13-week terminal sacrifices. These findings were most likely related to tissue trauma induced by multiple, i/v injections and were not related to the pharmacologic activity of the test article. Other observations included slight increases in the size of the spleen, multifocal, pale nodules in the liver, and parasitic nodules in the colon, cecum, and large intestine of several monkeys in all groups, including the control, at the 4- and 13-week sacrifices. There were no significant differences in either absolute, or relative organ weights for the monkeys treated with IFN-β, as compared to the control animals. Incidental findings included juvenile appearance of the testes, prostate, and epidiymides in many of the male monkeys, and decreased size of the ovaries in a few female animals at terminal sacrifices, indicating that these monkeys had not yet reached sexual maturity. These observations were confirmed upon histologic evaluation of the tissues, which demonstrated aspermatogenesis and aspermia in the male sex organs, and an immature, juvenile appearance to the prostate samples from the male monkeys, and the ovaries of several female animals.

On histologic evaluation, there were no pathologic findings that could be directly related to treatment with IFN-β. Chronic, inflammatory changes including predominantly

lymphoid infiltrates and aggregates were present in the stomach, small, and large intestines, pancreas, kidney, and urinary bladder of several monkeys in all groups, including the placebo control. These findings were not related to treatment with the test article, since they were present in approximately equal incidence and severity in all groups, and at both time points on study.

Microscopic findings in the liver included multifocal areas of hepatocellular degeneration and/or necrosis accompanied by mononuclear cell infiltrates, and slight to moderate inflammatory changes in the periportal regions. Vacuolization of hepatocytes, accompanied by evidence of fatty deposition in the liver were also present in several monkeys in all groups, including the controls. Histopathologic changes were also present in the lungs of animals in all groups, and included perivascular and peribronchiolar lymphoid cuffing, thickening of the pleural wall, and accumulation of pigment-laden macrophages. These findings were slight to mild in severity, occurred at approximately equal incidence between the groups and at the 4- and 13-week sacrifices, and were not considered related to IFN-β treatment.

Histologic examination of the injection site(s) from animals confirmed the gross pathologic findings of tissue trauma. Areas of perivascular and/or subcutaneous hemorrhage, perivascular inflammation, and fibrosis were present in monkeys in all groups, including the controls. Splenic changes included multifocal areas of congestion in the red pulp, in animals of all groups including the control. The findings in the spleens and injection sites were slight to moderate in severity, and were not related in either incidence or severity to the dose of IFN-β administered.

Analysis of serum levels of anti-HSA and anti-IFN-β antibodies demonstrated that after 4 weeks of treatment, increased titers of antibody to HSA had developed in the control and high-dose IFN-β male animals, as compared to the baseline values for these monkeys. Anti-HSA titers were not appreciably increased over baseline in the male monkeys receiving either 0.25 or 0.5 MIU/kg/d IFN-β, or in the control or high-dose females at this time point. Increases in antibody to HSA were apparent in the two groups of female monkeys treated with 0.25 or 0.5 MIU/kg/d IFN-β after both 4 and 13 weeks of treatment. The anti-HSA titers continued to increase over the duration of treatment, and were approximately 1.5 to 2-fold greater than baseline after 13 weeks of treatment, in both male and female animals. Interestingly, the values obtained for the anti-HSA titers in the male monkeys treated with 0.25 and 0.5 MIU/kg/d IFN-β were consistently lower than the antibody titers generated in the animals treated with the placebo control or with the highest dose of IFN-β.

Comment: As observed in the previous rat and monkey repeat-dose toxicity studies, this finding most likely results from the procedure used to dilute the samples prior to administration. Serial dilutions of IFN-β were done using 0.9% sterile saline, rather than the placebo control solution as the diluent. This practice effectively diluted the concentration of HSA in the mid-and low-dose groups by 50% and 25%, respectively, from what was present in the control and 1.0 MIU/kg/d IFN-β doses.

Antibody titers to IFN-β were elevated 21 to 41% after 4 weeks of treatment in all groups of monkeys receiving the biologic, as compared to the baseline values. In the female monkeys, anti-IFN-β titers did not increase significantly any further at the 3-week time points. By contrast, the male monkeys showed both dose- and time-related increases in anti-IFN-β titer, with increases of up to 1.5-fold over baseline observed in the group of male animals treated with 1.0 MIU/kg/d. The data are presented in the table, below:

Table XIV - Serum Antibody Titers in Cynomolgus Monkeys after Repeat I/V Administration of IFN-b

A. Male Monkeys

Dose of IFN-b (MIU/kg/d	**Mean Serum Antibody Binding Activity (cpm) ± S.E.M.**					
	Anti-HAS			**Anti-IFN-b**		
	Baseline	**4 weeks**	**13 weeks**	**Baseline**	**4 weeks**	**13 weeks**
0	953 ± 25	1050 ± 127	1010 ± 93[b]	6608 ± 256	6350 ± 180	5798 ± 398
0.25	863 ± 46	877 ± 43[a]	1455 ± 31[b]	7276 ± 382	8810 ± 230	9317 ± 195
0.5	996 ± 31	910 ± 61	918 ± 93[b]	6531 ± 254	8873 ± 402	9335 ± 161
1.0	963 ± 85	1653 ± 330	2191 ± 1082[b]	6329 ± 310	8777 ± 295	9676 ± 405
Serum blank	not done					

[a] n = 5 (one outlier)
[b] n = 3

B. Female Monkeys

Dose of IFN-b (MIU/kg/d	**Mean Serum Antibody Binding Activity (cpm) ± S.E.M.**					
	Anti-HAS			**Anti-IFN-b**		
	Baseline	**4 weeks**	**13 weeks**	**Baseline**	**4 weeks**	**13 weeks**
0	576 ± 19	616 ± 50	656 ± 57[a]	6217 ± 400	6054 ± 319	5632 ± 196
0.25	537 ± 17	799 ± 47	667 ± 87 [a]	6829 ± 410	9203 ± 107	8916 ± 96
0.5	691 ± 58	953 ± 192	1470 ± 346 [a]	6099 ± 354	8630 ± 156	8579 ± 298
1.0	589 ± 45	773 ± 117	6539 ± 94 [a]	5943 ± 335	8244 ± 198	8420 ± 291
Serum blank	not done					

[a] n = 3

In summary, daily i/v administration of IFN-β to cynomolgus monkeys was associated with only transient elevations in rectal body temperature. This is an expected, pharmacologic effect of type I interferons, and is not considered to be a toxic finding. There were no other overt clinical or gross pathologic signs of toxicity after up to 13 weeks of administration of the biologic. Antibodies to both interferon and the carrier protein were detected as early as 4 weeks in all groups of IFN-β-treated monkeys.

However, neutralizing antibody activity was not measured, so no conclusions regarding the effects of antibody development on the lack of toxicity can be made. The apparent NOAEL for IFN-β in the cynomolgus monkey after repeated, i/v injection for 4 or 13 weeks is $\geq$ 1.0 MIU/kg.

Study #XXXXXXXXXX. ***26-week repeated dose toxicity study in Cynomolgus monkeys treated with the test article REBIF administered by subcutaneous route at the doses of 0, 3.5, 10.5, and 35 mcg/kg/d.***

The long-term toxicities, toxicokinetics and pharmacodynamic effects, antibody development, and effects of IFN-β on reproductive parameters were evaluated in sexually mature, cynomolgus monkeys. Four animals of each sex received daily, s/c injections of vehicle control (sterile saline), 3.5, 10.5, or 35 µg/kg/d IFN-β for 6 months. These doses correspond to approximately 0, 1, 3, and 10 MIU/kg/d IFN-β activity, respectively. This study was designed to evaluate the safety of chronic administration of IFN-β by the intended, clinical route, in a species that is pharmacologically responsive to human, recombinant interferons.

Animals received daily, s/c injections of IFN-β into the subcutaneous tissue of the thigh, with injections alternated daily between the right and left sides. Body weights were obtained twice prior to the beginning of the treatment period, then at weekly intervals for the duration of the study. Adjustments to the amount of IFN-β administered were based on changes in weekly body weights, so that the dosing remained constant throughout the study. The test article was prepared by serial dilutions of IFN-β in the vehicle (0.9% saline).

Clinical observations were performed twice daily for mortality, and every day for signs of overt toxicity. Animals were allowed free access to an approximately 200 g ration of food per day, and supplemented with fresh fruit twice weekly. Filtered drinking water was available *ad libitum*, through an automatic watering system. Residual food was weighed and food consumption calculated, and expressed as g/animal/day. Water consumption was not measured.

Rectal body temperatures were determined using an electronic thermometer twice at baseline (days –5/-6, and immediately prior to dosing on d 0), and at 2 and 24 h following the first dose of IFN-β. Body temperatures were measured again at 2 and 24 h after dosing at weeks 4, week 13 and week 25, following the 22^{nd}, 85^{th}, and 169^{th} doses of IFN-β, respectively. Ophthalmologic examinations were performed on both eyes of each animal at baseline (week –2), and during the treatment period at weeks 13 and 26, prior to study termination. Electrocardiograms were not performed in this study. Urine samples for urinalysis profiles were collected from fasted monkeys after overnight housing in metabolic cages prior to treatment (day –5/-6), and at d 30/31 (week 5 on study), d 78 (week 12), and prior to study termination (d 176), after a water load of 20 ml/kg.

Peripheral blood samples for hematologic and serum biochemistry profiles were obtained at baseline (day –5/-6), week 5 (d 30/31), week 12 (d 78), and at week 26 (d 176), after the animals had been fasted overnight. Peripheral blood samples for analysis of serum IFN-β, as well as analysis of pharmacodynamic markers were also obtained from the monkeys prior to study initiation, then at weeks 1, 4, 13, and 25. One animal per sex was sampled at 1, 2, 3, and 4 h after administration of IFN-β, and then a second sample was obtained from each of these monkeys at 6, 8, 12, or 24 h, respectively. Serum samples were prepared, then stored frozen at –20°C until assayed for IFN-β levels by ELISA. Neopterin was measured as a surrogate, pharmacodynamic marker of IFN-β activity using an RIA on pooled samples from male and female monkeys taken 2, 6, and 24 h after the first IFN-β injection, and at weeks 1, 4, 13, and 25 as described above. The lower limit of detection of neopterin in the RIA assay was 0.5 ng/ml.

Serum obtained from these samples was also assayed for antibodies to IFN-β by ELISA. Anti-IFN-β neutralizing antibody activity was also determined from serum samples taken from one male and one female monkey in each experimental group, prior to initiation of treatment and at weeks 4 and 13, concomitantly with the blood samples for clinical pathology. Neutralizing antibody activity was determined based on the loss of IFN-β-induced, inhibition of the cytopathic effect of vesicular stomatitis virus (VSV) on human WISH cells. Antiviral activity induced by IFN-β, and its neutralization by serum from IFN-β treated monkeys was evaluated spectrophotometrically by determination of the optical density reading at XXXXXXXXXX, after staining of the viable, remaining WISH cells with crystal violet. The antibody titer was calculated as the reciprocal dilution of serum which reduced the IFN-β activity from the XXXXXXXXXX standard added to each well to XXXXXXXXXX (CPE_{50}). One LU is defined as a "laboratory unit," or the concentration of IFN-β which protects XXXXXXXXXX of the WISH cells from the cytopathic effects of VSV (CPE_{50}).

Additional blood samples were collected concomitantly with the samples for hematology and clinical chemistry, to evaluate the effects if IFN-β on serum testosterone (male) or estradiol (female monkeys) levels. Serum levels of sex hormones were evaluated using commercially available, RIA kits. Female monkeys were observed daily for evidence of onset of menses; cyclicity and duration of menses were also evaluated in control and IFN-β-treated female animals.

Semen samples were collected from anesthetized male monkeys by electroejaculation prior to initiation of IFN-β treatment (days –26 to –12), and at weeks 4, 13, and 26. Sperm counts, motility, and morphology were determined microscopically, using computer-assisted analysis for the motility assay. The functionality of the sperm from IFN-β-treated male monkeys was also evaluated using the *in vitro*, zona-free hamster oocyte penetration test. This assay measures the ability of the sperm to penetrate and decondense their DNA within hamster oocytes (*i.e.* fertilize) after a 3 h incubation period.

At terminal sacrifice, the body weights were recorded for each individual animal after an overnight fast. Animals were euthanized by an overdosage of 50 mg/kg sodium

pentobarbital, i/v, exsanguinated through the femoral arteries, and subjected to a complete necroscopic examination. Gross pathologic evaluation and organ weights were measured for all monkeys, and samples of target organs preserved in either10% buffered formalin or XXXXXXXXXX solution (eyes, testes, epididymides), embedded in paraffin, and stained for histopathologic examination at a later date.

Body weight data, food consumption, clinical pathology and urinalysis profiles, rectal body temperature, sperm motility, count, morphology, and function and organ weights were analyzed statistically for differences between the control and the three IFN-β-treated groups by one-way ANOVA, or where appropriate by non-parametric analysis with a XXXXXXXXXX ANOVA. Further differences within the groups were analyzed by XXXXXXXXXX (parametic) or the XXXXXXXXXX (non-parametric). The level of significance for all tests was $p \leq 0.05$, except where indicated ($p \leq 0.01$).

There were no mortalities on study, and no overt signs of clinical toxicity in any of the groups of monkeys treated with IFN-β. No ophthalmologic changes were noted in any animals in either the control or the IFN-β-treated groups. One male monkey (animal #498M, 35 µg/kg/d IFN-β) developed a rectal prolapse during week 3 on treatment, which was manually reduced by the examining veterinarian. This finding was considered a spontaneous event which is occasionally reported in untreated monkeys as well, and therefore was considered unrelated to treatment with the test article. Alopecia of the fore- or hindlimbs, back, and/or trunk was present on several animals, including those in the control group, and was not related to IFN-β treatment. Local irritation, as evidenced by scratching at the injection sites and reluctance to treatment was observed in monkeys treated with 10.5 or 35 µg/kg/d IFN-β, beginning after approximately two weeks on study and lasting for the remainder of the treatment period.

Although the female monkeys in the highest IFN-β dose group (35 µg/kg/d) demonstrated slight decreases in group mean body weight as compared to either baseline or to vehicle control animals during the first month of treatment, these differences were not statistically significant. Food consumption by these monkeys was also decreased, although not significantly, during this time period. This group of animals continued to maintain average body weight values for the remainder of the study that were decreased by approximately 6% from baseline mean value. There were no effects of IFN-β treatment on overall body weight in either the low- or mid-dose female monkeys, or in any of the groups of male monkeys on study. Similarly, there were no treatment-related effects on food consumption in any of these groups, although incidental findings of increased food consumption were reported in the high-dose males at week 10, and in females of all IFN-β-treated groups at week 21 on study.

There were no statistically significant, treatment-related changes in hematologic or serum biochemistry profiles in any of the male monkeys treated with IFN-β, as compared to animals receiving the vehicle control. Incidental findings included higher group mean values for urea at week 5 and α1-globulin at week 12, with a lower mean γ–globulin fraction at week 26 for males in the high-dose group when compared to the controls. Although the differences were statistically significant, further analysis revealed they were

due to corresponding decreases in the mean values for the control group at these time points, and did not reflect significant changes from baseline values. These findings were not considered to be of toxicologic relevance to the IFN-β treatment.

By contrast, female monkeys treated with either 10.5 or 35 μg/kg/d IFN-β showed statistically significant increases in neutrophil counts with concomitant decreases in lymphocyte counts at week 12 on study, as compared to the vehicle control groups. Although not statistically significant, total leukocyte counts were also decreased at all time points on study for all of the IFN-β-treated groups of female monkeys, when compared to the vehicle control group. However, these changes did not always represent decreases from baseline values; the apparent findings may have resulted from unusually high values for the control females, and are of no toxicologic significance. Erythrocyte counts, hemoglobin, and hematocrit levels demonstrated slight, occasionally significant fluctuations over the duration of the study, as compared to either the vehicle control or baseline values. The only statistically significant changes were a 14% decrease in hemoglobin levels for females in the high-dose groups at week 26, and a 28-32% decrease in platelet levels in the low-and mid-dose females at week 5, as compared to control animals. A slight increase in prothrombin time was also observed for the female monkeys treated with 10.5 μg/kg/d at week 12. However, this finding was not significantly different from control at the 26 week time point, and therefore was not considered related to treatment with the test article.

Overall, serum biochemistry profiles in the female monkeys were not significantly different from either control or baseline values following treatment with IFN-β. However, prior to study initiation, serum AST levels were significantly ($p \leq 0.01$, ANOVA) lower than control value in the group of animals planned to receive 3.5 mg/kg/d IFN-β, and were significantly lower than control values ($p \leq 0.05$, ANOVA) for the two groups of female monkeys treated with 10.5 or 35 μg/kg/d IFN-β. Treatment with IFN-β did not affect serum AST levels any further over the 26-week study course. Other findings in the female animals included a slight (< 10%) depression of total protein levels in the mid-dose group at weeks 12 and 26 ($p < 0.01$, XXXXXXXXXX) and a 28-33% increase in α1-globulins at week 5 in the mid- and high-dose groups, respectively, as compared to the vehicle control ($p < 0.01$, XXXXXXXXXX). A transient increase in α2- globulin, as compared to both baseline and vehicle control values was observed for female monkeys in the group treated with 35 mg/kg/d at week 5 only ($p \leq 0.05$, ANOVA). Decreases in the γ-globulin fraction, as compared to either baseline or control values were present in the mid- and high-dose females throughout the study duration, and reached statistical significance ($p \leq 0.05$, XXXXXXXXX) by week 26. None of these findings were felt to be related to treatment with IFN-β, as there was no clear dose-response relationship, and the changes were considered of no toxicologic significance.

There were no significant, treatment-related or toxicologically relevant changes in urinalysis profiles from either male or female monkeys treated with IFN-β, as compared to animals treated with the vehicle control. Several female monkeys in all groups, including the control, had presence of blood in the urine at various time points on study.

These findings corresponded to onset of menses in these animals, and were not related to treatment with IFN-β.

Semen analysis profiles from samples obtained from male monkeys treated with IFN-β did not reveal any treatment-related differences from control in the parameters measured, including sperm counts, morphology, percentage of motile and static sperm, velocity, track speed, beat frequency and lateral amplitude, path velocity, straightness, or linearity at any time point measured. The one exception was that the number of viable sperm per ml ejaculate was always lower, although not significantly, than either the control or the baseline values in the males in the group treated with 10.5 µg/kg/d IFN-β. This finding was significantly decreased ($p \leq 0.01$, XXXXXXXXXX) from both the control and the baseline group findings at the week 13 time point, but had partially resolved by the end of treatment period at week 26. An approximate 5-fold increase ($p \leq 0.05$, ANOVA) in sperm counts per ml ejaculate was observed at week 26 for the monkeys treated with 3.5 µg/kg/d IFN-β, as compared to the vehicle control group. However, this finding, although not statistically significantly different from control, was consistently observed at all earlier time points for this group of animals, including prior to initiation of IFN-β treatment and is not considered biologically significant.

There were no effects of IFN-β treatment on sperm function, as determined using the *in vitro*, hamster oocyte penetration test. The mean percentage of oocytes penetrated by sperm from IFN-β-treated monkeys ranged from 9.75 to 41% at baseline, with approximately 1.5 decondensed sperm/oocyte. These values were not appreciably affected after 4 weeks of treatment. At 13 weeks, the mean values of both percent of oocytes penetrated as well as the mean number of decondensed sperm per oocyte had decreased to zero for the group of monkeys treated with 10.5 µg/kg/d IFN-β. The mean percentage of penetration had decreased in all groups, including the control by about 50% from the baseline values at this time point. These values had further decreased in all groups, including the control at study termination at 26 weeks. The sponsor attributes these findings to a failure to produce enough ejaculate by the electroejaculation procedure at these time points, and does not consider the transient absence of penetration at week 13 in the mid-dose monkeys to be related to treatment with the test article. Serum testosterone levels were unaffected, as compared to either baseline values or to the vehicle control group, by treatment with IFN-β.

There were no significant differences in either the duration of menses or the time in each cycle in any of the groups of IFN-β-treated female monkeys, as compared to the female animals treated with the vehicle control. For the time points measured, there were no significant differences between the groups in serum estradiol levels, and no apparent effects of IFN-β treatment on ovulation. Occasionally, an apparent increase in the mean values was observed in one or more of the groups; this was usually related to an elevation in estradiol in a single female in that group, suggesting onset of ovulation.

Serum interferon levels were below the limits of detection of the assay (< 5 IU/ml) in all of the monkeys prior to study initiation, and in all control animals at all time points, with the exception of monkey #508F, who had an elevation in serum IFN-β activity to 5 U/ml

at 2 h after the week 4 injection on d 22. Since these animals were not followed individually for toxicokinetics, it is not possible to say if this finding was real or an artifact of either the collection or assay procedure(s). Serum interferon levels were detectable in all groups of monkeys after the initial dose on d 1, with C_{max} obtained approximately 3-4 h after injection, and related to the dose of IFN-β administered. By 4 weeks on study, no IFN-β activity could be detected in serum from the low-dose animals, with the exception of monkey #514F, who had a level of 6 U/ml 4 h after injection. Serum IFN-β levels, although highly variable, were detectable in the 4/8 male and 3/8 female animals in the 10.5 µg/kg/d dose group, and in 1/8 male and 4/8 female monkeys in the high-dose group after 4 weeks of treatment. Serum levels of IFN-β were undetectable at any time point after injection in all four groups of monkeys at 13 or 26 weeks. The loss of detectable IFN-β activity was correlated with the development of both total and neutralizing antibody activity (please see below).

Neopterin levels were elevated in all groups of IFN-β-treated monkeys at 24 h following the first dose of the test article. By 4 weeks on study, there was no significant increase in the pharmacodynamic marker in the groups treated with either 3.5 or 10.5 µg/kg/d IFN-β, and the response in the high-dose group was blunted and appeared earlier than that observed after the first study dose (9.8 pg/ml at 6 h after the d 22 injection, as compared to 25.6 ng/ml at 24 h after the initial injection). By week 13, no stimulation of neopterin was observed in any of the treatment groups. Again, these findings were correlated with the development of anti-IFN-β neutralizing activity, and suggest a loss of both the pharmacologic and toxicologic properties of the agent.

Serum antibody and neutralizing antibody activities were increased in all groups of animals treated with IFN-β, beginning at week 4 and continuing throughout the duration of treatment. Interestingly, the total anti-IFN-β antibody titers did not increase significantly after the 4 week time point, and there was no apparent relationship of the titer of antibody induced to the dose of IFN-β administered. Test samples for determination of week 26 antibody titers were evaluated separately from the remainder of the samples; therefore, a second baseline value is included for the purpose of comparison. The results are presented in the two tables below:

Table XV - Serum Antibody Titers in Cynomolgus Monkeys after Repeat S/C Administration of IFN-b

A. Male Monkeys

Dose of IFN-b **(**m**g/kg/d)**	**Mean Serum Antibody Binding Activity (OD_{490}) ± S.D.**				
	Baseline	**5 weeks**	**12 weeks**	**Baseline**	**26 weeks**
0	50 ± 33	54 ± 36	50 ± 29	104 ± 31[a]	186 ± 102
3.5	37 ± 11	1478 ± 91	1510 ± 145	139 ± 41	1483 ± 159
10.5	42 ± 15	1312 ± 271	1422 ± 186	95 ± 41	1563 ± 172
35.0	39 ± 15[a]	1437 ± 53	1518 ± 8	134 ± 59[a]	1671 ± 107

[a] n = 3 (one outlier)

B. Female Monkeys

Dose of IFN-b (mg/kg/d)	Mean Serum Antibody Binding Activity (OD_{490}) ± S.D.				
	Baseline	**5 weeks**	**12 weeks**	**Baseline**	**26 weeks**
0	115 ± 66	131 ± 88	117 ± 78	217 ± 91	196 ± 74
3.5	96 ± 49[a]	1534 ± 96	1664 ± 66	158 ± 59[a]	1484 ± 45
10.5	179 ± 90	1533 ± 35	1634 ± 61	189 ± 75	1567 ± 115
35.0	143 ± 89	1475 ± 54	1579 ± 88	199 ± 59	1550 ± 234

[a] n = 3 (one outlier)

Neutralizing antibody titers were also increased in serum samples taken from one representative animal per sex at each time point. No neutralizing antibody activity was detected in samples from any monkey prior to initiation of treatment, or in either of the two control monkeys at any time point on study. By 5 weeks on study, male monkey #459M in the low-dose group had a slight positive, anti-IFN-β titer (32, 20 neutralizing units [NU]/ml), while the female monkey in this group as well as the two mid-dose animals had undetectable levels of neutralizing activity at this time point. By contrast, a marked anti-IFN-β titer (7988, 2902 NU/ml) was present in the male monkey treated with 35 µg/kg/d IFN-β, while the female monkey from this group had a slightly positive (31, 20 NU/ml) titer at the 5 week time point. By 12 weeks on study, the neutralizing antibody titers were present in all 6 of the IFN-β-treated monkeys, at levels between 100 to 1000 NU/ml (monkey #478F, low-dose, 802 NU/ml; monkey #446M, mid-dose, 138 NU/ml; and monkey #474F, high-dose, 397 NU/ml), to titers of > 1000 NU/ml in the remaining three animals (monkey #459M, low-dose, 1288 NU/ml; the antibody titers for the remaining two animals in the mid- and high-dose groups exceeded the highest dilution range [1:4096] tested). These neutralizing antibody levels correlated with a loss of both detectable serum IFN-β activity, as well as the loss of the pharmacodynamic marker, neopterin at these time points (see above).

There were no treatment-related findings on gross pathological examination of the IFN-β-treated monkeys at the time of necropsy (27 weeks). There were no significant differences in either absolute or relative organ weights for the monkeys treated with IFN-β, as compared to the control animals. Areas of hemorrhage in the subcutaneous tissue at the injection site(s) were present in several animals in both the control and the IFN-β-treated groups, and were related to tissue trauma induced by multiple, s/c injections. Other observations included whitish to pale nodules in the liver, and parasitic nodules in the lymph nodes, urinary bladder, lungs, colon, cecum, and large intestine of several monkeys in all groups, including the control. On histologic evaluation, there were no pathologic findings that could be directly related to treatment with IFN-β. Chronic, inflammatory changes including predominantly lymphoid infiltrates and aggregates were

present in the stomach, gall bladder, small and large intestines, pancreas, kidney, and urinary bladder of several monkeys in all groups, including the vehicle control. These findings were not related to treatment with the test article, since they were present in approximately equal incidence and severity in all groups, and at both time points on study.

Microscopic evaluation of the liver revealed multifocal areas of hepatocellular degeneration and/or necrosis accompanied by mononuclear cell infiltrates, and slight to moderate inflammatory changes in the periportal regions. In some cases, thickening of the capsule overlying the areas of inflammation was also present. Several animals had multifocal aggregates of mononuclear cells present in the gallbladder; phenotypically, these cells appeared to be lymphocytic in origin. Vacuolization of hepatocytes, accompanied by evidence of fatty deposition in the liver were also present in several monkeys in all groups, including the controls. Microscopic changes in the lungs were also present in animals from all treatment groups, and included perivascular and peribronchiolar lymphoid cuffing, thickening of the pleural wall, and accumulation of pigment-laden macrophages. These findings were slight to mild in severity, occurred at approximately equal incidence between the groups, and were not considered related to IFN-β treatment.

Histologic examination of the injection site(s) from animals confirmed the gross pathologic findings of tissue trauma. Areas of perivascular and/or subcutaneous hemorrhage, perivascular inflammation, and fibrosis were present in monkeys in all groups, including the controls. These changes were slight to mild in severity, and were not related either in incidence or severity to the dose of IFN-β administered. Sternal bone marrow showed multifocal areas with changes consistent with atrophy and replacement by fat cells, which is not unusual for monkeys of this age. Fatty atrophy was also present in the thymus of many of the animals in all treatment groups, including the control, and is not an unexpected finding for animals in this age range.

In summary, treatment of male and female cynomolgus monkeys for 6 months with 3.5, 10.5, or 35 µg/kg IFN-β was associated with transient weight loss and inappetence, slight anemia, and changes in leukocyte differential profiles in the female monkeys at the highest dose. There were no other overt, gross pathologic or histologic findings, which indicate toxicity due to repeated dosing with the biologic. Serum IFN and neopterin levels were detectable after the first dose in all groups of IFN-β-treated monkeys, and only in animals in the highest dose group after 4 weeks of treatment. Antibody development, both as total IgG and as anti-IFN-β neutralizing activity was evident in animals in all IFN-β treated groups by 5 weeks on study. Total anti-IFN-β antibody activity was not related to either the dose or duration of IFN-β treatment, as similar titers were achieved in all three groups of monkeys receiving the test article at either 5, 12, or 26 weeks on study. By contrast, low titers of anti-IFN-β neutralizing activity were present in ½ low-dose monkeys and 2/2 animals in the highest dose group at 5 weeks, and continued to increase over the duration of the study. At the last time point measured at 12 weeks on study, anti-IFN neutralizing titers were > 1000 NU/ml in 3/6 monkeys, and between 100 and 1000 NU/ml for the remaining 3 animals. The increase in anti-IFN

neutralizing antibody was correlated with a loss of both detectable serum levels of IFN-β, as well as a decrease in induction of neopterin levels. There was no relationship of neutralizing antibody titer developed to the dose of IFN-β administered.

Based on the loss of appetite, subsequent weight loss, and hematologic changes in the female monkeys, the NOAEL of IFN-β administered for 6 months by daily s/c injection is 10.5 µg/kg/d (3 MIU/kg/d). The NOAEL in male monkeys is 35 µg/kg/d (10 MIU/kg/d), given by s/c injection for 6 months. These doses correspond to approximately 70 to 245 times the cumulative weekly dose of 66 µg IFN-β used in the pivotal trial (as calculated for a 60-70 kg human), and 35 to 122 times the cumulative weekly dose of 132 µg. When scaled by total body surface area, these doses are approximately 28 to 100-fold greater than the cumulative human weekly dose of XXXXXXXXXX IFN-β proposed for use in relapsing-remitting multiple sclerosis.

Study #XXXXXXXXXXX. ***Local irritation study in rabbits treated with the test article REBIF (finished product) by the intramuscular route.***

The local irritation effects of IFN-β were evaluated in New Zealand white rabbits after a single i/m injection. Three animals per group, per time point were dosed with 0.5 ml of either 0.425% or 1.7% acetic acid or IFN-β (12 MIU/ml), i/m into the right *vastus lateralis* muscle group. The contralateral side was injected with 0.5 ml of sterile, 0.9% saline as a vehicle control. Macroscopic observations for local irritation were performed daily after injection, and any findings were rated numerically on a score of 0 to 4 for evidence of local irritation, erythema, and/or edema. Rabbits were euthanized following the final observations on days 2 and 14; the injection sites examined for evidence of gross pathologic lesions, and preserved in 10% buffered formalin. However, since the severity of irritation induced by IFN-β on gross pathologic evaluation was determined as no different from control, the samples were not examined microscopically.

All animals survived until study termination, and there was no clinical evidence of overt toxicity related to IFN-β treatment. Rabbit #141 (group 1, IFN-β and saline-injected) and rabbit #155 (1.7% acetic acid and saline-injected) both developed hematomas at the site of vehicle injection. The hematomas were slight (rabbit #155) to moderate (rabbit #141) in severity, and were related to trauma induced by the injection, and not to treatment with the test article at the site.

Macroscopically, one rabbit in the IFN-β-treated group exhibited very slight (grade 1) hemorrhage and local irritation at both the IFN-β and the saline injection sites after 2 days of observation. The area of involvement of hemorrhage was approximately 24 mm^2 for the IFN-β injected site, as compared to 71 mm^2 at the saline-injected site 48 h after treatment. No gross evidence of inflammation, hemorrhage, or local irritation was observed in any of other rabbits in this group at either the 2-d or 14 d sacrifices. By contrast, all three rabbits sacrificed at 2 d after injection of 1.7% acetic acid had severe (Grade 3) hemorrhage and white discoloration (edema) at the injection site, which had only partially resolved to moderate severity by d 14. The affected areas ranged from 425

to 800 mm^2 on d 2, to 60 to 176 mm^2 on d 14 for the animals in this group. Samples were not evaluated histologically for evidence of microscopic lesions.

In conclusion, intramuscular injection of 0.5 ml of either 0.9% sterile saline or 6 MIU formulated IFN-β was not associated with any, acute, local irritation effects. A single animal had findings consisting of localized areas of hemorrhage (Grade 1 severity) at both the saline control and IFN-β-injected sites. These effects are due to the acute trauma associated with the injection procedure, and do not appear to be test article-related. The NOAEL for local irritation of IFN-β after i/m injection is therefore 6 MIU given in 0.5 ml sterile saline, or approximately equal to the dose used in the clinical trials in multiple sclerosis.

Study #XXXXXXXXXX. ***Evaluation of teratogenic and abortifacient potential of interferon beta recombinant in the cynomolgus monkey.***

The effects of IFN-β treatment on reproductive and developmental toxicity were evaluated in pregnant cynomolgus monkeys after dosing during either the critical period of organogenesis (GD21 – GD89) or during late pregnancy (GD90 until term, approximately GD150). Six gravid female monkeys per group were treated with 0.2, 0.6, or 1.8 MIU/kg/d of interferon or placebo control by i/m injection during the period of organogenesis from GD21 – GD89. Dose volume was held constant at 0.25 ml/kg, and doses adjusted as needed based on changes in weekly body weights. An additional six monkeys/group received IFN-β or placebo control at the same doses by i/m injection from days 90 until the infants were vaginally delivered at term (approximately GD150), for a total of 60 doses.

At GD100, surviving fetuses from animals treated during organogenesis were obtained by Caesarean delivery, and subjected to standard teratologic evaluations including physical measurements, whole body, organ, and placental weights, amniotic fluid volumes, and gross evaluation of the viscera, including the brain. Following gross evaluation, all tissues and carcasses were preserved in appropriate fixative, stained with XXXXXXXXXX, and evaluated for skeletal and soft tissue abnormalities. Live offspring born to dams treated from GD90 to term were examined for physical abnormalities, and the sex, body and placental weights, and limb measurements from each infant were recorded.

Animals were also evaluated for signs of maternal toxicity by assessing physical signs and body weights, rectal body temperature, and hematologic profiles. Clinical observations were conducted twice daily. Rectal body temperatures were obtained under ketamine anesthesia prior to dosing and at 4 h post-treatment on GD21 and GD88 for animals treated during organogenesis, and at the same time points on GD90 and GD149 for the monkeys treated with IFN-β during late pregnancy. Blood samples for determination of hematologic and clinical chemistry profiles, and for evaluation of anti-IFN-b antibody development were obtained prior to dosing on GD20, then on GD35,

GD50, and GD88 for monkeys treated during the critical phase of organogenesis, and on GD120 and GD149 for animals treated from GD90 until term.

Maternal animals in all eight groups had no clinically evident signs of toxicity related to treatment with IFN-β. Body weights, body weight gains, appetite, and behavior and appearance were all within normal limits throughout the treatment period, and no maternal deaths occurred on study. Occasional incidences of soft stools and/or diarrhea were observed in animals in all treatment groups, including the controls, and were not considered related to IFN-β toxicity. Other clinical findings included infrequent observations of emesis, vomitus present underneath the cage floor, blood and/or mucous in the stool, and animals with an oily appearance to their coat. These changes were considered incidental to treatment with IFN-β, since they occurred in all groups of animals, including the controls.

There were no apparent effects of treatment with IFN-β on rectal body temperature in any of the pregnant dams. Hematologic changes included a slight increase in hemoglobin as compared to control on GD 50 in the monkeys treated with 0.2 MIU/kg/d IFN-β, and a 30-35% decrease in platelet counts on GD 35 in the animals treated with the high dose from GD21-GD89 ($p < 0.05$, ANOVA). Findings in the animals treated late in pregnancy (GD90-GD150) included statistically significant increases in erythrocyte parameters (hemoglobin and hematocrit levels) and a decrease in the absolute neutrophil count in monkeys in the highest dose group on GD120, as compared to the control group. All of these changes, however, had resolved to control levels by the next time point on study, and were not considered to be toxicologically relevant.

Comment: The International Congress on Harmonization (ICH) has recently adopted a set of guidelines for the conduct of reproductive toxicity studies in preclinical animal models. In this document, it is specified that the highest dose of test article used in these types of studies should induce significant maternal toxicity, which IFN-β did not. However, abortions were incurred after 20 to 30 doses with 0.2 MIU/kg IFN-β (see below); these events have been adequately represented in the package insert information. No further segment II or segment III reproductive toxicity testing with this material will be required for licensure.

In the animals treated with the test article from GD21 until GD89, one animal each in the groups treated with 0.2 or 0.6 MIU/kg/d IFN-β was found to have spontaneously aborted on GD40 and GD51, respectively, as determined by ultrasound. No fetal tissues were recovered so evaluation was not possible, and the exact date of fetal loss could not be determined. There were no abortions or loss of conceptus in either the control group or the pregnant dams treated with 1.8 MIU/kg/d IFN-β during the period of organogenesis.

An increased incidence of fetal loss and stillbirths was observed in the dams treated with IFN-β from GD90 until term. Two animals in the group treated with 0.2 MIU/kg/d IFN-β aborted during the dosing period, on GD134 (monkey #F1691 and #F49267). Two additional monkeys in this group delivered stillborn offspring (conceptus #90-707, on

GD150 and conceptus #91-288, on GD160). One infant in the placebo control group and one infant in the monkeys treated with 0.6 MIU/kg/d IFN-β were delivered stillborn on GD154 and GD161, respectively. Gross evaluation of the aborted or stillborn fetuses did not reveal any external malformations. There were no prenatal losses in the group of monkeys treated with 1.8 MIU/kg/d IFN-β, either during the period of organogenesis, or during late gestation until term. However, one infant in this group was delivered live on GD153 (#F1F, male) but died 4 days after birth of unknown causes. Necropsy evaluation revealed dark and reddened areas in the lungs, however, the post-mortem autolysis was extensive, and no definitive cause of death could be determined.

Statistically, there were no significant differences in the abortion rate between IFN-β-treated and control animals, or when compared to historical control rates for cynomolgus monkeys housed at a similar animal facility (please see comment, below).

Comment: The study report cites abortion/stillbirth rates between 1983 and 1990 for indoor-housed, long-tailed macaques (cynomolgus monkeys) at the California Regional Primate Research Center as a 3.5% loss rate (4/113) in vehicle control animals and a 17.2% loss rate (34/198) in untreated control monkeys, during the early part of gestation (up until GD100). For a full-term pregnancy, fetal loss rates are estimated at 3.5% (4/113) and 25% (50/198) for vehicle and untreated control monkeys, respectively. Compared to these figures, the rate of fetal loss in the control and 0.6 MIU/kg/d IFN-β dose groups are not significantly different. However, the increased incidence of both abortions and stillbirths in the lowest dose group (4/6, or 67% loss) is significantly elevated over the expected rates. Since abortifacient effects of other interferons in this class have previously been documented, a treatment-related effect cannot be disregarded.

In fetuses recovered by Caesarean section on GD100, there were no apparent, gross fetal malformations in any of the offspring that could be attributed to treatment with either the vehicle control, or with any dose of IFN-β. No differences in fetal body and/or organ weights, or external physical measurements were detected in fetuses from any of the IFN-β-treated dams, as compared to the control animals. Placental membrane, amniotic fluid and cord measurements in the IFN-β-treated groups were not significantly different from the respective measurements in the control animals. Several placental variations were observed in fetuses from all of the treatment groups, including controls, and included fatty depositions throughout the placenta and membranes, fusion of primary and accessory lobes, calcification on the fetal surface adjacent to the umbilical cord, and some cyst-like structures seen in patches on the fetal surface. Three animals in the high-dose group had alterations in the umbilical cord, consisting of swelling from the point of fetal insertion to approximately halfway up the cord. A similar thickening of the umbilical cord, although not reported as swelling, was observed in one fetus in the control group as well. These findings are all normal variations for this strain of monkey and were not considered related to the test article.

Several minor skeletal changes were noted in fetuses from the dams treated with either vehicle control or IFN-β during the period of organogenesis; however, these effects were all considered to be within normal limits for cynomolgus monkeys bred in captivity, and

were considered unrelated to interferon treatment. One of the aborted fetuses in the group treated with 0.2 MIU/kg/d from GD90-GD150 (conceptus #90-707) had multiple skeletal abnormalities in the thoracic vertebrae, including unilateral (1^{st}, 4^{th}, and 7^{th}), asymmetric (2^{nd} and 3^{rd}), fused (3^{rd} vertebral centrum fused to the 4^{th}) and bilobate vertebrae (6^{th} thoracic vertebrae). This first rib on the left side of this animal was also small. Since formation and development of the spinal vertebrae and the ribs occurs early during gestation and prior to initiation of IFN-β treatment, these findings were not felt to be related to the test article.

There were no apparent soft tissue anomalies in fetuses from dams receiving interferon from GD21 through GD89. Several minor variations in fetal organ weights were observed in fetuses from dams treated with IFN-β when compared to the controls, including slightly increased group mean values for adrenal weights in the groups treated with 0.2 or 0.6 MIU/kg/d IFN-β. There was no apparent dose-relationship in either incidence or severity of soft tissue variations, and no statistically significant differences between groups. These minor defects were felt by the evaluating pathologist to be within normal limits for captive-bred macaques.

In summary, treatment of pregnant cynomolgus macaques with 0.2 MIU/kg/d IFN-β resulted in spontaneous abortions and/or fetal losses in 1/6 monkeys treated from GD21 through GD89, and in 4/6 monkeys treated during late pregnancy (GD90 through term). Although not statistically significantly different from either vehicle or historical control values, these effects are consistent with the abortifacient effects of other type I interferons. No fetal malformations or other evidence of teratogenesis were noted, and no significant maternal toxicities were incurred. Because a higher incidence of fetal loss occurred at the lowest dose of IFN-β tested, no NOAEL for toxicity to reproduction can be determined.

Comment: There were no data included in the final report regarding the specific activity of the IFN-β material used in this study. The doses used in the animals were recalculated and compared to the human dose, using the specific activity stated in the label of 0.27 MIU/µg protein. When these values were used, the cumulative weekly dose of approximately 1.1 µg/kg in a 60 kg human is approximately 5-fold lower than the lowest dose in the animals that induced the abortifacient effects. The higher human dose of 44 µg/t.i.w. in the humans is approximately 2.4-fold lower than the dose of IFN-β which caused significant fetal loss in the animals.

When the doses were compared based on MIU of anti-viral activity present in the clinical formulation for marketing as compared to the material used in this preclinical trial, the cumulative weekly doses in the human were 0.3 or 0.6 MIU/kg. The cumulative, weekly dose in the animals that induced significant fetal loss was 1.4 MIU/kg, or approximately 4-fold greater than the weekly human dose of 66 µg, and 2-fold higher than the human dose of 132 µg/week.

SUMMARY AND CONCLUSION:

The safety, biochemical, and pharmacologic activity of IFN-β, derived from CHO cells were evaluated in mice, rats, and cynomolgus monkeys *in vivo*, and in peripheral blood leukocytes derived from humans, mice, rats, rabbits, dogs, and cynomolgus monkeys *in vitro*. In *in vitro* pharmacodynamic assays, only PBL from cynomolgus monkeys were found to exhibit increases in 2',5'-OAS enzyme activity similar to that observed with human cells; a dose-related increase in serum neopterin activity was also observed when PBL from cynomolgus monkeys were incubated with IFN-β *in vitro*, or in serum samples from animals treated *in vivo.* Because of the species specificity in the pharmacodynamic response, *in vivo* pharmacologic, pharmacokinetic, and repeat-dose toxicology testing was conducted in rats and in the cynomolgus monkey. Pharmacokinetic studies in these species demonstrated similar absorption and elimination after either s/c or i/m injection, with a "flip-flop" profile observed in both species following injection of 1 MIU/kg IFN-β. Systemic exposure, as calculated from the C_{max} and $AUC_{0-\infty}$ were increased in a dose-related fashion, was approximately linear although greater than predicted, and were similar for both the i/m and s/c routes. Bioavailability by either route was approximately 25 to 40%. IFN-β has pharmacologic and toxicologic profiles similar to other type I interferons; major findings in cynomolgus monkeys after repeated s/c dosing at 0.1, 0.25, 1 or 10 MU/kg (0.3, 1.25, 5, or 50 µg/kg) of IFN-β included slight increases in rectal body temperature, decreased food consumption and weight loss, slight variations in erythrocyte, platelet, and leukocyte counts, and local irritation and inflammation at the site of injection. There was no apparent dose-relationship of any parameter to the dose of IFN-β administered, and the majority of these changes were only evident during the first two to four weeks of treatment. Histologically, increases in lymphoid hyperplasia, chronic inflammation, and hemorrhage were observed at the injection sites after 4 to 26 weeks of treatment, which were also present in animals treated with the vehicle control. The NOAEL for IFN-β in both rats and in cynomolgus monkeys was 1.0 MIU (0.37 µg)/kg/d for 4 or 13 w, by either i/v or i/m injection. Based on the loss of appetite, subsequent weight loss, and hematologic changes in the female monkeys, the NOAEL of IFN-β administered for 6 months by daily s/c injection is 10.5 µg/kg/d (3 MIU/kg/d). The NOAEL in male monkeys is 35 µg/kg/d (10 MIU/kg/d), given by s/c injection for 6 months. These doses correspond to approximately 70 to 245 times the cumulative weekly dose of 66 µg IFN-β used in the pivotal trial (as calculated for a 60-70 kg human), and 35 to 122 times the cumulative weekly dose of 132 µg. When scaled by total body surface area, these doses are approximately 28 to 100-fold greater than the cumulative human weekly dose of XXXXXXXXXX IFN-β proposed for use in relapsing-remitting multiple sclerosis.. Pharmacodynamic markers of interferon activity, including 2 to 50-fold increases in serum neopterin and 2',5'-OAS levels were observed in animals after single doses of IFN-β, but declined after treatment for more than 28 days, and levels returned to baseline after 4 to 13 weeks on study. A loss of detectable IFN-β activity in the serum and development of neutralizing antibody activity was noted at the end of treatment period in all studies, beginning by approximately 4 weeks of treatment.

IFN-β was tested for reproductive and developmental toxicity in male and female cynomolgus monkeys as part of the 26-week toxicity study, and was found to have no effect on serum estradiol levels or menstrual cyclicity at doses of up to 10 MIU/kg/d. In male monkeys, there were no significant, treatment related effects of IFN-β treatment on male fertility, as evidenced by serum testosterone levels, sperm counts, motility, and function (hamster oocyte penetration) at doses of up to 10 MIU/kg/d (35 µg/kg/d). Treatment of pregnant, female cynomolgus monkeys from either GD21-89 pr GD90-150 induced significant abortifacient effects at 0.2 MIU (0.74 µg)/kg/d, with a 67% fetal loss rate when animals were treated late in pregnancy. This dose level is approximately 2 to 4-fold greater than the recommended weekly dose of 66 µg (18 MIU) or 132 µg (36 MIU) in MS patients, when normalized by either body weight or surface area.

The data adequately represent the pharmacology, pharmacokinetics, and toxicities associated with type I interferons, and support the safety of this product for licensure for use in multiple sclerosis. The abortifacient effects of IFN-β have been clearly represented in the proposed package insert, and the biologic has been assigned a pregnancy category "C", which is appropriate for these data.

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Key Words: interferon-β, multiple sclerosis, toxicology

concurrences:

OTRR/C,P-T/MGreen

cc:

OTRR/C,P-T/MGreen